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# TEXAS REPORTS ON BIOLOGY and MEDICINE

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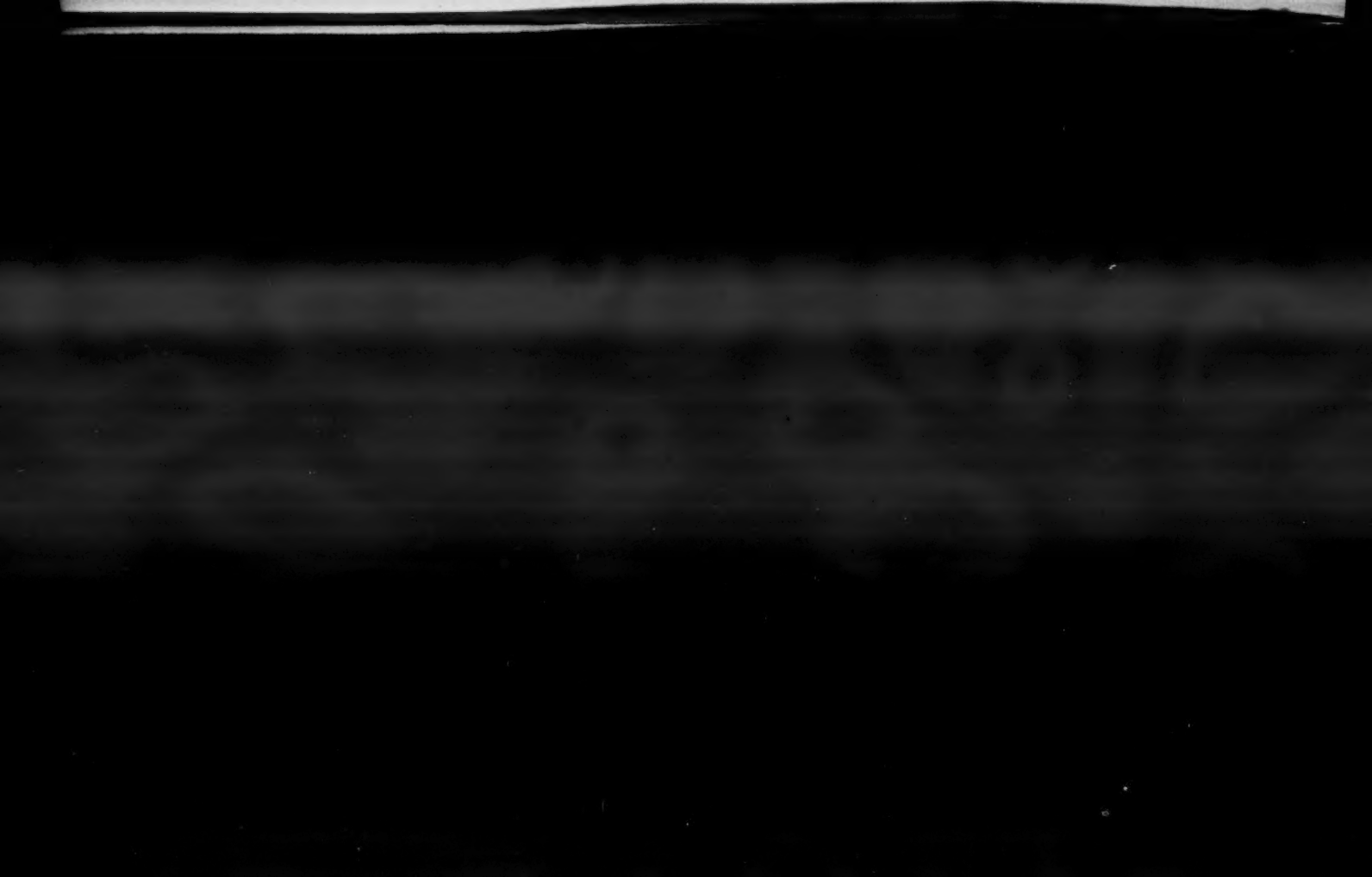

# ON THE ROLE OF THE ADRENAL CORTEX IN THE METABOLISM OF AMINO ACIDS*

JORGE AWAPARA
WITH THE ASSISTANCE OF WINNIFRED SKELLENGER AND NANCY MANZ

The participation of the adrenal cortex in protein metobolism was well demonstrated by the experiments of Long, Katzin, and Fry (1946). They observed glycogen deposition in the liver, but not in the muscle of rats and mice after the administration of adrenocortical extracts; at the same time they noticed that the nitrogen excretion was also increased in these animals. The increase in the excretion of urinary nitrogen was assumed to be the result of breakdown of protein which served as precursor for the extra glycogen, but proof was not given. Since the appearance of Long's paper a number of investigations have been reported, in which attempts have been made to clarify the mechanism by which the adrenal cortex affects protein metabolism and gluconeogenesis. Many of them have been reviewed by Long (1941).

If the two phenomena, namely increased nitrogen excretion and increased glycogen deposition in the liver, were directly related, then it is possible for the adrenal cortex to act at any of the several steps involved in the conversion of protein to glycogen. At first, the adrenocortical hormones were thought to be essential in the deamination of amino acids, but no definite proof has ever been reported. Russell and Wilhemi (1941) using kidney slices from adrenalectomized animals demonstrated that glycogen formation from glutamic acid and from alanine was less than normal. Samuels and associates (1936) had previously reported that glycogen formation from fed DL-alanine was less than normal in the adrenalectomized rat. But Evans (1941) could find no difference in the rates at which DL-alanine disappeared from the blood of adrenalectomized or intact rats. Lewis *et al.* (1940), reported the results of an extensive study in

* From the Division of Protein Chemistry, The University of Texas M. D. Anderson Hospital and Tumor Institute, Houston. This investigation was supported by grants from the National Cancer Institute, National Institutes of Health (C-1831-C) and from the American Cancer Society.

which adrenalectomized, phlorizinized rats were used. They showed that the conversion of alanine and pyruvate to glucose was less than normal in adrenalectomized rats; they offered the alternative interpretation that glucose was oxidized faster in these animals.

Albright (1943) suggested that the cortical hormones exert their effect by inhibiting protein synthesis; thus the amino acids not used for protein could then be used for glycogen. In a series of papers, Engel and associates (Engel, Schiller, and Pentz, 1949; Bondy, Engel, and Farrar, 1949; Engel, 1951) report results of experiments designed to prove that cortisone had no effect upon the rate of urea formation and by inference that cortisone inhibited protein synthesis. It is somewhat unfortunate that a comparison of results obtained in various laboratories is often impossible because of the variety of techniques and animals used. We cannot assume that the effects elicited by the administration of cortisone are, or should be the opposite of effects observed following adrenalectomy. Nor can we assume that all the effects elicited by the administration of cortisone are directly related.

It is clear now from the work of Dougherty and White (1944) that cortisone causes atrophy of the lymphatic structures. This is an indisputable fact and could well account for the extra nitrogen excretion observed so often after the administration of cortisone. There is always the possibility that extra nitrogen excretion and increased liver glycogen deposition are two distinct events that occur simultaneously. With all the available information it is still impossible to know how cortisone acts and how it causes glyconeogenesis from protein.

This investigation was started in the hope that some existing discrepancies could be cleared; the experiments reported here were not designed to yield conclusive and final results, but were conducted as orienting experiments that could lead to further and more fruitful investigations. Three aspects of the problem were studied and the results are reported here: (1) the changes if any, in the concentration of free amino acids in the liver following adrenalectomy and cortisone treatment; (2) possible changes in transaminating activities of liver, thymus and spleen following adrenalectomy, adrenal replacement, and cortisone

treatment, and (3) the rates of utilization of injected alanine and serine by intact and adrenalectomized rats. Utilization in this case is taken to mean the same as disappearance of the administered amino acid from the blood and tissues. Studies on L-alanine have been reported (Awapara and Kit, 1954) and in this paper we will only present results obtained for L-serine.

## *Experimental*

*Changes in the Concentration of Hepatic Free Amino Acids after Adrenalectomy.* Wistar rats weighing approximately 200–250 grams were used. Bilateral adrenalectomy was performed in these animals, under light ether anesthesia. The procedure used needs no description since it is a standard laboratory operation. The adrenalectomized animals were divided into two groups of five each. All of them were given salt in the drinking water and food *ad libitum*. After one week allowed for recovery, the animals were subjected to a 48 hour fast. During this time, 5 mg. of cortisone acetate per day was given to each rat of one group; the other group received a corresponding amount of physiological salt solution. As controls, two groups of intact rats treated in the same manner as the adrenalectomized animals were used. At the end of the experimental period, the animals were sacrificed, the livers then removed, aliquots weighed and protein free filtrates prepared for assay according to the method of Schurr *et al.* (1950). Ten amino acids were determined microbiologically and the results shown in table 1. It is clear from the table that only insignificant changes occur; the only change of any significance is in the histidine content of the liver from adrenalectomized rats.

TABLE 1

*Changes in Concentration of Endogensus Amino Acids Following Adrenalectomy*

(All values are in micrograms amino acid per gram fresh tissue; glycogen in mg. per 100 gm.)

| Determined | Intact Saline | Intact Cortisone | Adrenalectomized Saline | Adrenalectomized Cortisone |
|---|---|---|---|---|
| Glycogen | 2.3± 0.45 | 26.7± 2.2 | 0.40± 0.07 | 12.3± 0.8 |
| Isoleucine | 32± 1.5 | 35± 1.8 | | |
| Phenylalanine | 33± 2.7 | 39± 3.1 | 38± 1.3 | 39± 2.2 |
| Valine | 50± 5.6 | 66± 6.0 | 63± 5.3 | 68± 5.2 |
| Leucine | 72± 8.8 | 97± 7.4 | 100± 4.8 | 92± 5.4 |
| Lysine | 96± 6.4 | 131± 4.7 | 106± 5.8 | 136± 3.1 |
| Tyrosine | 44± 3.0 | 45± 2.6 | | |
| Histidine | 28± 1.4 | 35± 1.4 | 80± 3.8 | 47± 4.8 |
| Threonine | 88±17.5 | 116±10.0 | 68± 9.9 | 53±10.9 |
| Serine | 132± 7.7 | 113± 5.1 | 126± 5.0 | 169± 8.5 |
| Glycine | 275±11.1 | 319± 7.2 | 240± 5.1 | 262± 9.2 |

All values are the arithmetical means plus or minus the standard error (five animals per group).

The adrenalectomized animals receiving cortisone vary little from the intact receiving cortisone. This is difficult to assess.

In a second series of experiments some amino acids and related compounds were measured by means of paper chromatography. In this case, female rats were used and amino acids measured in both liver and skeletal muscle. The extracts were prepared according to a previously reported method (Awapara, 1948) and chromatographic analysis carried out according to a recently modified procedure (Kit and Awapara, 1953). The results are shown in table 2.

TABLE 2

*Changes in Concentration of Endogensus Amino Acids Following Adrenalectomy*

(All values are given in micromoles per gram fresh tissue)

| Treatment | Asp | Glu | Ala | Tau | PE* | Gly-Ser |
|---|---|---|---|---|---|---|
| Intact, Liver | 1.0 | 2.5 | 2.4 | 7.6 | 1.4 | 2.7 |
| ( ) | (0.7–1.4) | (2.1–2.8) | (1.8–2.9) | (6.1–10.1) | (1.3–1.6) | (2.2–3.2) |
| Adrenalectomized Liver | <1.0 | 2.2 | 1.6 | 5.3 | 2.3 | 2.2 |
| ( ) | .... | (2.0–2.3) | (1.3–1.8) | (3.3–7.5) | (1.5–3.3) | (1.9–2.5) |
| Intact, Muscle | «1.0 | «1.0 | 4.3 | 13.8 | «1.0 | 3.4 |
| ( ) | .... | .... | (3.4–4.8) | (11.8–16.8) | .... | (3.0–4.0) |
| Adrenalectomized Muscle | «1.0 | 1.7 | 2.9 | 11.4 | «1.0 | 2.2 |
| ( ) | .... | (1.3–1.9) | (2.0–3.5) | (10.4–12.6) | .... | (1.8–2.4) |

( ) Range
* Phosphorylethanolamine

The most striking difference is in the content of phosphorylethanolamine (also called, among other names, ethanolamine phosphoric acid), which increases in the liver after adrenalectomy of the animals. This has been observed in our laboratory sometime ago and consistently it has been found that this compound is significantly higher in the livers of adrenalectomized rats than in the normal liver. Since little is known about the origin of the compound or its metabolism, no explanation can be given at the moment for this occurrence.

Phosphorylethanolamine was identified in practically every organ of the rat (Awapara, Landua, and Fuerst, 1949) and exists in very high amounts in the spleen. The other hepatic amino acids change little; in the muscle there is a definite decrease in the concentration of alanine and glycine-serine (measured together) and an increase in glutamic acid.

*Transaminating Activities of Liver, Spleen, and Thymus in Adrenalectomized and Cortisone Treated Rats.* The methods for the estimation of alanine-glutamic transaminase and aspartic-glutamic transaminase have been reported in a previous paper (Awapara and Seale, 1952). Again, four groups of animals were used and the conditions were the same as those indicated in

the first part of this study. In addition to transaminase activity, total nitrogen was determined by a micro-Kjeldahl procedure. The results of this study are shown in table 3. In the liver, there are no apparent differences in the

TABLE 3

*Effect of Adrenalectomy on the Transaminating Activities of Liver, Spleen and Thymus**

| Treatment | LIVER N† | LIVER Ala | LIVER Asp | SPLEEN N | SPLEEN Ala | SPLEEN Asp | THYMUS N | THYMUS Ala | THYMUS Asp |
|---|---|---|---|---|---|---|---|---|---|
| Intact Saline | 38.4 | 484 (440–520) | 320 (290–360) | 32.5 | 28 (22–32) | 270 (254–276) | 28.8 | 42 (26–60) | 242 (206–310) |
| Intact Cortisone | 37.8 | 428 (340–500) | 304 (300–360) | 29.5 | 60 (22–60) | 275 (200–300) | 21.2 | 52 (46–64) | 304 (290–320) |
| Adrenal-ectomized Saline | 38.2 | 380 (300–400) | 316 (260–380) | 30.4 | 26 (10–32) | 272 (200–300) | 28.6 | 22 (10–40) | 180 (120–200) |
| Adrenal-ectomized Cortisone | 34.2 | 426 (400–465) | 282 (236–320) | 30.6 | 23 (19–65) | 292 (240–320) | 27.4 | 28 (16–45) | 220 (190–250) |

* Average of four animals and range of variation values are given in micromoles of glutanic acid formed per gram of fresh tissue per hour.
† In mg. per 100.

transaminase activity of any of the groups. Unfortunately some of the values are so low that any differences become questionable. Furthermore, the individual variation, if taken into account, could explain most of the differences observed in the table. The same can be said for the nitrogen content. It is of interest that the lymphatic tissues change but little in their transaminating activity even though they have suffered a considerable loss of weight and in the thymus of the cortisone treated rat, a considerable loss of nitrogen.

*Utilization of L-Serine by Adrenalectomized and Intact Rats.* In these experiments five pairs of adrenalectomized and five pairs of intact rats were used. The conditions and methods of analysis were the same as those described for alanine (Awapara and Kit, 1954), except that a two-hour group was included. Four organs were analyzed: liver, kidney, heart, and spleen. The results as shown in tables 4a, 4b, 4c, and 4d, indicate that there are some

TABLE 4a

*Utilization of Injected L-Serine by Normal and Adrenalectomized Rats*
(All values are given in micromoles per gram fresh tissue)

LIVER

| Time | Ser-gly C | Ser-gly Ad | Ala C | Ala Ad | PE* C | PE* Ad | Glu C | Glu Ad | Asp C | Asp Ad |
|---|---|---|---|---|---|---|---|---|---|---|
| 0 | 2.0 | 1.8 | 2.9 | <1.0 | 1.4 | 3.2 | 2.3 | 1.2 | 1.0 | <1.0 |
| 15 | 12.8 | 9.0 | 10.9 | 11.2 | 1.4 | 3.6 | 4.2 | 5.6 | 1.8 | 2.8 |
| 30 | 5.2 | 15.8 | 3.5 | 9.0 | <1.0 | 3.2 | 3.0 | 3.0 | 1.2 | 3.0 |
| 60 | 4.1 | 10.0 | 3.9 | 2.6 | 1.2 | 3.3 | 1.5 | 4.1 | 1.0 | 2.7 |
| 120 | 3.0 | 3.0 | 2.9 | <1.0 | <1.0 | 3.7 | 2.2 | 1.2 | <1.0 | <1.0 |

* Phosphorylethanolamine.

TABLE 4b

KIDNEY

| Time | Ser-gly | | Ala | | PE* | | Glu | | Asp | |
|---|---|---|---|---|---|---|---|---|---|---|
| | C | Ad | C | Ad | C | Ad | C | Ad | C | Ad |
| 0 | 4.9 | 3.6 | 1.2 | <1.0 | 2.8 | 3.1 | 6.0 | 4.9 | 1.5 | 2.0 |
| 15 | 23.5 | 19.5 | 2.4 | 1.5 | 2.1 | 1.5 | 7.5 | 7.6 | 2.0 | 1.3 |
| 30 | 12.6 | 17.0 | 1.5 | 1.4 | 2.6 | 2.2 | 9.0 | 6.7 | 1.9 | 1.7 |
| 60 | 8.3 | 10.0 | 1.7 | 1.6 | 2.3 | 2.5 | 7.0 | 6.5 | 1.8 | 1.4 |
| 120 | 6.0 | 9.0 | 1.9 | 1.1 | 2.5 | 2.7 | 7.3 | 7.5 | 1.4 | 1.3 |

* Phosphorylethanolamine.

TABLE 4c

HEART

| Time | Ser-gly | | Ala | | PE*† | | Glu | | Asp | |
|---|---|---|---|---|---|---|---|---|---|---|
| | C | Ad | C | Ad | C | Ad | C | Ad | C | Ad |
| 0 | 1.0 | 1.5 | 3.1 | 2.8 | .. | .. | 5.5 | 6.7 | 1.5 | 1.4 |
| 15 | 4.5 | 4.2 | 2.4 | 3.0 | .. | .. | 5.2 | 5.5 | 1.3 | 1.3 |
| 30 | 4.5 | 4.5 | 2.8 | 3.3 | .. | .. | 4.2 | 6.6 | 1.2 | 1.0 |
| 60 | 3.5 | 4.5 | 2.9 | 3.3 | .. | .. | 6.0 | 5.0 | 1.2 | 1.0 |
| 120 | 3.5 | 3.6 | 2.7 | 2.6 | .. | .. | 4.4 | 5.8 | 1.3 | 1.3 |

* Phosphorylethanolamine.
† Concentration too low to be determined accurately.

TABLE 4d

SPLEEN

| Time | Ser-gly | | Ala | | PE* | | Glu | | Asp | |
|---|---|---|---|---|---|---|---|---|---|---|
| | C | Ad | C | Ad | C | Ad | C | Ad | C | Ad |
| 0 | 1.7 | 1.5 | 2.2 | <1.0 | 6.8 | 3.5 | 4.9 | 2.4 | 3.5 | 1.5 |
| 15 | 5.1 | 6.0 | 1.2 | 1.0 | 6.7 | 6.2 | 3.7 | 3.8 | 2.0 | 2.0 |
| 30 | 4.8 | 7.6 | 2.0 | 2.1 | 7.0 | 5.0 | 4.7 | 2.5 | 2.6 | 1.3 |
| 60 | 4.2 | 9.0 | 1.0 | 1.8 | 5.4 | 8.0 | 4.2 | 3.9 | 2.6 | 1.4 |
| 120 | 3.6 | 3.1 | 1.2 | 1.3 | 4.0 | 4.6 | 4.7 | 3.0 | 2.5 | 1.6 |

* Phosphorylethanolamine.

differences in the rates at which serine disappears from the liver of adrenalectomized rats as compared to the intact rats. Unfortunately serine and glycine cannot be resolved by paper chromatography and there is no way of knowing how much of the area of the paper represents serine. In both adrenalectomized and intact rats there is a rapid conversion to alanine and the extent of this conversion is about the same in both groups. Moreover at the end of two hours the alanine concentration in the liver returns to the basal level in both groups; the concentration of phosphorylethanolamine does not change at any time, but as before it is much higher in the liver of adrenalectomized rats. Glutamic acid increases in the liver, just as if alanine had been injected. In the other organs we could not find any significant changes.

*Discussion.* The hormones from the adrenal cortex, cortisone in particular, appear to cause an increase in the free amino acids of muscle but not in liver. In the liver only glutamic and aspartic acids are affected: they decrease in concentration. This was previously reported (Awapara, Marvin, and Wells, 1949), and confirmed by Dubreuil and Timiras (1953). The so-called catabolic action of the adrenal cortex has been known for some time, but the exact mechanism has never been explained. Our results show that the most conspicuous change is in the concentration of phosphorylethanolamine; this compound invariably increases in concentration after adrenalectomy. No explanation can be given at present for this significant change, but work has been planned to study the metabolism of this compound. The decrease in the concentration of both glutamic and aspartic acids could be correlated with the increased glyconeogenesis. The proximity of these amino acids to the Krebs' cycle makes them the most likely precursors for carbohydrate.

The fact that amino acids are set free by cortisone, and that glycogen is formed in the liver, does not necessarily mean that there is a cause and effect relationship, since amino acids are readily available when cortisone is influencing glycogen formation, it seems plausible that they should be utilized for that purpose. If some other precursor of glycogen were made available at that time, the amino acids set free could probably be spared.

Engel (1951) demonstrated that small doses of glucose tended to depress the formation of the extra urea which is formed after ACTH administration; larger doses of glucose, he reported, could depress urea formation from injected amino acids. This evidence supports in part the view that the two events, *i.e.* glycogen formation and the excretion of extra nitrogen could be independent. Our own results on utilization of alanine by adrenalectomized rats (Awapara and Kit, 1954) indicate that there is no defective deamination in those animals, at least insofar as alanine is concerned. The rapid removal of the pyruvate (probably formed from injected alanine) from the blood in the adrenalectomized rats could be explained in several ways, but all explanations would be speculative.

The important fact that emerges from these results is that the

action of the adrenals must be exerted beyond the deamination stage. Our data also shows that in any experiment in which an amino acid is given intravenously disappearance from the blood can only be established by more than one measurement. By measuring several amino acids at once it is possible to detect interconversions such as those observed after the injection of alanine and serine. In all of our experiments only the L-isomers were used; thus the results obtained were free of the complications which arise when recemic mixtures are used. If all these factors are taken into account we could understand some of the apparent discrepancies which exist in the literature.

Transaminating activities in liver, thymus, and spleen do not change significantly after various forms of adrenal treatment. From those results we can conclude that there is no effect on either the alanine-glutamic transaminase or aspartic-glutamic transminase after adrenalectomy. A partial confirmation was obtained *in vivo*, where glutamic acid is formed from alanine in about the same amounts in both adrenalectomized and intact rats. The conversion of serine into alanine, by whatever mechanism, also appears to remain undisturbed in the adrenalectomized rat.

In the thymus and spleen there are no changes in transaminase activity even though these organs had lost considerable weight after administration of cortisone; transaminase activity is expressed in terms of unit weight and since no changes were discernible after cortisone treatment we can assume that the destruction of these tissues is uniform. The same does not apply to organs such as the prostate in which the alanine transaminase activity decreases when that organ loses weight following castration (Awapara, 1952) or the liver which loses nitrogen by feeding the animals a protein free diet (Awapara, 1953).

It appears that the adrenal cortex exerts more than one type of metabolic effect; some of these effects can and do elicit secondary actions not necessarily related to the adrenal cortex. The extra glycogen deposition and extra excretion of nitrogen after the injection of cortisone or adrenocortical extracts appear to be two phenomena not necessarily related; no proof of this can be furnished as yet, but much of the evidence favors this view.

*Summary*

The effects of adrenalectomy and cortisone administration on the concentration of free amino acids in the liver of the rat were investigated. No changes could be observed in ten amino acids measured microbiologically. The only changes observed were in the concentration of glutamic and aspartic acids and alanine. An unexpected increase in the concentration of phosphoryl-ethanolamine was observed in the liver of adrenalectomized rats.

Alanine-glutamic and aspartic-glutamic transaminases were measured in the liver, thymus, and spleen of adrenalectomized rats and controls. Simultaneously, total nitrogen was determined. No correlation could be found between loss of nitrogen in the thymus and spleen with transaminase activity. The probable reasons for this apparent lack of effect were discussed. In the liver there were no changes in transaminase activity.

The utilization of L-serine in adrenalectomized and control animals was investigated. Injected L-serine into rats appears as such in the liver within a short time after the injection. The alanine content and in some instances the glutamic acid content of the liver was found to increase after serine injection. This conversion was apparently not affected by adrenalectomy.

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# NOCARDIOSIS: DISCUSSION OF TWO CASES WITH EMPHASIS ON DIAGNOSTIC FEATURES*

MARY P. CLAPP AND MARJORIE J. WILLIAMS

The term nocardiosis usually refers to infection caused by *Nocardia asteroides* and its variants. *N. madurae* and *N. intracellularis* and a few other species also produce disease (Cuttino *et al.*, 1949; Conant *et al.*, 1954); only one instance of infection due to *N. intracellularis* has been recorded (Cuttino *et al.*, 1949). *N. intracellularis*, however, differs in many respects from the type species. *N. asteroides* is a member of the family *Actinomycetaceae*. It is aerobic as opposed to the better known anaerobic member, *Actinomyces bovis* (Breed *et al.*, 1948; Weed and Baggenstoss, 1949). As a general rule, the disease starts as a persistent localized infection and, unless arrested by treatment, may disseminate.

Approximately 65 cases have been recorded, but the incidence may be greater than this would indicate. In many of these cases, the diagnosis was elusive during life and was established only after death by culture and by histologic study of autopsy material. This suggests that spontaneous healing may occur in some cases without the true nature of the disease having been detected. As nocardiosis in its early stages is reported to be amenable to treatment with sulfonamides, it is imperative that the diagnosis be made as soon as possible (Benbow *et al.*, 1944; Connar *et al.*, 1951; Runyon, 1951; Strauss *et al.*, 1951; Conant, 1954; Conant *et al.*, 1954). Unfortunately this is frequently rather difficult as the disease may resemble many other chronic infections, particularly tuberculosis (Glover *et al.*, 1948), and even neoplasms (Krueger *et al.*, 1954). Further, the organisms are not invariably present in materials submitted for examination and culture.

Although *N. asteroides*, and hence the disease, is world-wide in distribution, it is only in recent years that a significant num-

* From the Laboratory Service, Veterans Administration Center, Temple, Texas. Part of this material has been incorporated in an exhibit entitled Nocardiosis, which was presented at the Second International Congress of Clinical Pathology, Washington, D.C., 1954.

ber of cases has been reported. As familiarity with both the clinical picture and the characteristics of the etiologic agent should facilitate diagnosis, we are reporting two cases with primary pulmonary nocardiosis occurring in residents of Central Texas. Prior to this, one case occurring in Texas has been recorded (Eckhardt and Pilcher, 1950).

Both of our cases are somewhat similar. The patients were young white men in their late twenties. Each ran a prolonged course with ultimate dissemination and a fatal outcome. In both, the infection was initially restricted to the lungs.

*Report of Cases*

*Case 1.* A 28-year-old white man had been under the care of his local physician for about 15 months prior to admission to the hospital. The initial symptoms, which led him to seek medical advice, were those of a chronic bronchitis with a productive cough and intermittent fever. Clinical and radiologic studies were considered to indicate a fungus infection of the lungs. However laboratory investigations failed to reveal the etiologic agent. He was treated with iodides and various antibiotics, with no amelioration of his symptoms. Shortly before admission, he developed severe persistent headaches and his physician recommended hospitalization.

Examination at this time showed a chronically ill young man with low grade fever and slight mental clouding. The chest was clear on auscultation and percussion but an X-ray demonstrated a dense infiltration containing a cavity at the left hilus. Neurologic examination was negative except for slight haziness of the optic disc margins. A spinal puncture was performed and the cerebrospinal fluid escaped under increased pressure (235 mm. $H_2O$); the fluid was clear and colorless with 110 white blood cells per cu. mm. (50 per cent polymorphonuclear leukocytes, 50 per cent lymphocytes), 143 mg. per cent of protein and 80 mg. per cent sugar. No organisms were detected in smears of the fluid. An electro-encephalogram indicated a focal abnormality in the left temporal lobe.

While in the hospital, the patient's general condition deteriorated progressively, with increasing mental confusion and terminal coma. Therapy with sulfadiazine and actidione was undertaken on the assumption that there was a fungus infection of the lungs with cerebral metastases. However these measures produced no remission, and the patient expired two weeks after hospitalization.

At autopsy, the following pertinent findings were demonstrated. Around the hilus of the left lung there was a greyish-yellow zone of induration with central cavitation. In the left temporal lobe there was a large irregularly-shaped abscess, which had ruptured into the inferior horn of the lateral ventricle. The pus was light green in color and was odorless. The overlying meninges were hyperemic but showed no other evidence of involvement.

By study of direct smears, it was demonstrated that the pus from the brain

abscess contained filamentous and coccobacillary organisms, which were gram-positive and variably acid-fast (Fig. I). *N. asteroides* was cultured from the pus. No significant organisms could be recovered from the pulmonary lesion; cultures for *Mycobacterium tuberculosis* were negative. *N. asteroides* was also recovered from sputum cultures but these did not become positive until after death.

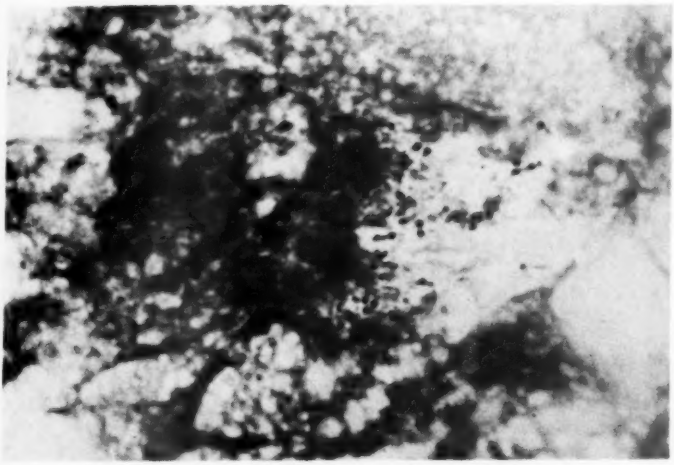

FIG. I. Smear of pus from brain abscess demonstrating coccobacillary forms of *N. asteroides*. X 2900.

Microscopic study demonstrated a granulomatous pneumonitis at the left hilus. Multi-nucleated giant cells of the Langhans type were present, but caseation was absent. The brain abscess was surrounded by encephalomalacia and gliosis but there was no granulomatous inflammation. In hematoxylin and eosin stained sections organisms were not detected in either lesion. However it was possible to demonstrate in sections of the brain abscess bacillary and filamentous structures, which were gram positive and variably acid-fast (Fig. II).

A final diagnosis was made of nocardial brain abscess probably secondary to pulmonary nocardiosis.

*Case 2.* The second case illustrates more forcibly the similarities between nocardiosis and tuberculosis and the ease with which the former disease may be mistaken for the latter. The patient was a 29-year-old Latin American man, who became ill nine months prior to admission to the hospital. The initial symptom was sharp stabbing pain in the right shoulder. He reported this to his company doctor, who ordered a chest X-ray and, on the basis of the radiologic findings, referred him to a hospital to determine whether pulmonary tuberculosis was present. Sputum and gastric washings were both

negative for *M. tuberculosis*. For the next three months, the patient felt fairly well, but at the end of this period he developed low grade fever and reported to a physician again. On his advice the patient was admitted to another hospital some six weeks later. Despite intensive studies undertaken there, a definitive diagnosis was not made. On one occasion acid-fast bacilli were detected in a sputum smear but culture of this specimen was negative for *M. tuberculosis*. By radiographic means, a mass was demonstrated in the right retroperitoneal region. At this time, however, the patient elected to leave the hospital against medical advice.

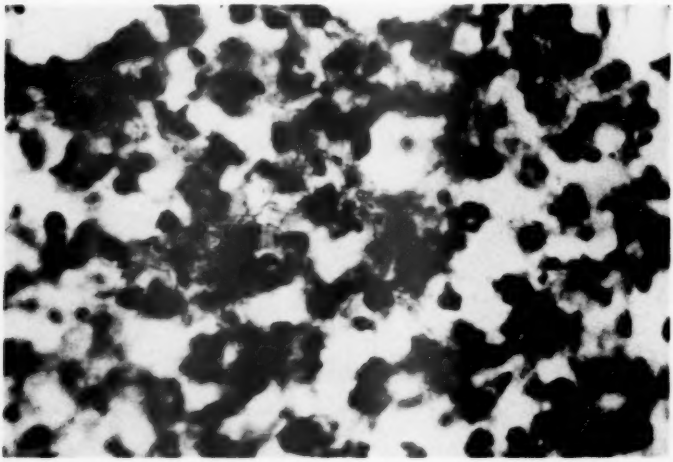

FIG. II. Section of brain abscess demonstrating primarily filamentous forms of *N. asteroides*. X 2000.

He remained at home for approximately four weeks. During this time he was febrile with a severe productive cough and some dyspnea. He was then admitted to this hospital.

On admission, he was emaciated and was considered to be severely ill; he had a temperature of 103° F., a pulse rate of 100 per minute and a respiration rate of 30 per minute. Examination of the chest demonstrated an area of dullness at the right base and coarse rales bilaterally. The liver and spleen were slightly enlarged and there was tenderness over the liver. A chest X-ray showed extensive patchy bilateral pneumonitis.

Laboratory studies demonstrated a leukocytosis with neutrophilia and a slight albuminuria. Study of direct smears of sputum was not contributory. Penicillin therapy was initiated but the patient deteriorated rapidly and died one week after admission.

Autopsy studies revealed a severe patchy suppurative pneumonitis involving both lungs. In the right retroperitoneal space was a tremendous abscess

extending from the diaphragm to the pelvis. In addition there was thrombophlebitis of the inferior vena cava. The brain showed no change.

Examination of smears of pus from the retroperitoneal abscess demonstrated gram positive and occasionally acid-fast filamentous organisms. *N. asteroides* was recovered in cultures of blood from the right atrium, tissue from both lungs and pus from the retroperitoneal abscess. *M. tuberculosis* could not be isolated from this material.

Microscopic study demonstrated granulomatous lesions in both lungs (Fig. III), in the wall of the retroperitoneal abscess and in the thyroid. In all areas

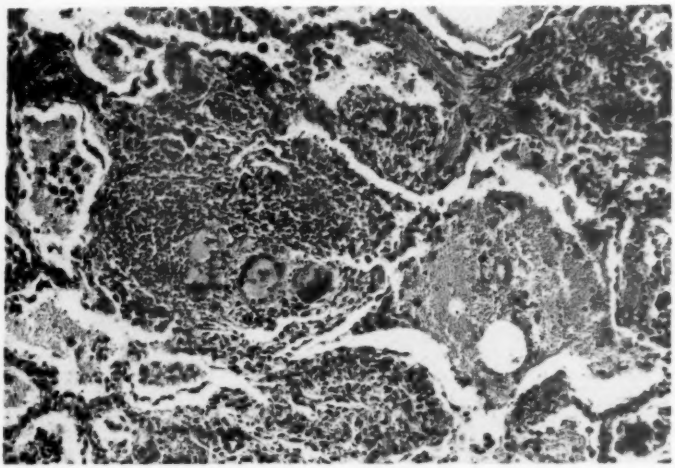

FIG. III. Section of nocardial granuloma in the lung demonstrating giant cells of the Langhans type. X 180.

other than the thyroid, many of the granulomas had undergone central suppuration (Fig. IV). In the lung, rupture of granulomas into pulmonary veins was observed. By means of the gram stain bacillary and filamentous forms were found in the lesions. These organisms were not acid-fast in tissue sections.

A diagnosis of pulmonary nocardiosis with dissemination was made.

*Cultural Studies.* Since *N. asteroides*, as a disease agent, is not frequently encountered in the laboratory and since in both our cases the organism was atypical, it seems desirable to describe in some detail the organism recovered from these cases and the methods used for its identification. In each instance the colony on blood agar was chalky white, rough and firmly adherent to

the medium (Fig. V). The classical colony is yellow to red in color, generally smooth, and easily removed from the medium by a platinum loop (Breed *et al.*, 1948). Examination of the literature shows that the organisms recovered in many of the reported cases more closely resembled ours than those described in Bergey's Manual. (Benbow *et al.*, 1944; Binford and Lane, 1945; Kirby and McNaught, 1946; Jacobson and Cloward, 1948; Hager *et al.*, 1949; Tucker and Hirsch, 1949; Cullen and Sharp, 1951; Bernstein *et al.*, 1952; Cruz and Clancy, 1952; Kaufman and Prieto, 1952; Wichelhausen *et al.*, 1954).

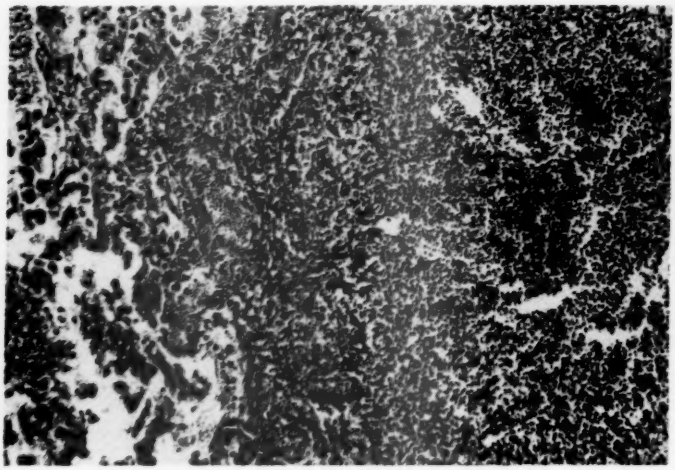

FIG. IV. Section of nocardial granuloma in the lung demonstrating central suppuration. X 200.

The organism grew readily, although relatively slowly as it required approximately 4 days to form easily visible colonies on blood agar at 37° C. Growth was best under aerobic conditions, was somewhat inhibited by an atmosphere of 10 per cent $CO_2$, and was absent under strict anaerobiasis. The organism would grow at room temperature only slowly and only if a large inoculum were used. Nutrient agar and Sabouraud's agar also supported growth but not as luxuriant as on blood-enriched media. The colonies on solid media showed a slight indentation of the agar surrounding them. The periphery of such a colony, sus-

pended in saline and slightly crushed, showed it to be composed of a mass of filaments, when examined under the microscope (Fig. VI). Careful search revealed some true branching forms.

Growth in litmus milk and in gelatin occurred as discrete, chalky white, dry, round colonies floating on the surface. There were no changes in litmus milk after 60 days, but the organisms became quite uniformly acid-fast. Gelatin was not liquefied.

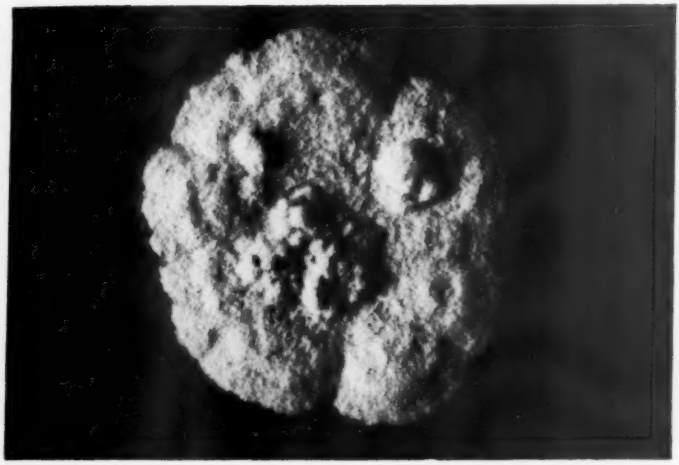

FIG. V. Giant colony of *N. asteroides*. X 14.

The virulence of each strain for laboratory animals was checked. Rabbits were inoculated by the intravenous route and guinea pigs intraperitoneally. After three weeks none of the animals showed any clinical signs of infection. At this time the rabbits were sacrificed and autopsied. All organs were grossly normal, but were cultured irrespective of their appearance. *N. asteroides* was recovered from the spleen and the adrenals of the rabbit inoculated with the strain from our second case. The other strain was not recovered. The guinea pigs have not yet shown any signs of infection, but have not been sacrificed up to this time (9 months). Appearance of lesions in mice has been reported as late as 6 months following inoculation (Cruz and Clancy, 1952).

*In vitro* tests of sensitivity to the following antibiotics were performed: penicillin, aureomycin, chloromycetin, terramycin, streptomycin, erythromycin, neomycin, aerosporin (polymixin B). The organism was found to be sensitive to aureomycin and to terramycin but not to any of the others.

FIG. VI. Wet preparation demonstrating filamentous periphery of colony. X 1150.

*Comment.* These two cases in conjunction with one another illustrate fairly well the clinical aspects of visceral nocardiosis. In 62 recorded cases, pulmonary lesions were present in 40, cerebral lesions in 15, and involvement of the subcutaneous tissues in 21 (Wichelhausen *et al.*, 1954). Almost all other organs have been reported as affected but the incidence of infection in such sites is small (Wedding, 1947; Wichelhausen *et al.*, 1954). Some instances of subcutaneous tissue infection and of osteomyelitis have occurred following trauma (Cullen and Sharp, 1951). Either pulmonary disease or subcutaneous abscesses are usually the first manifestations (Conant *et al.*, 1954). There are occasional cases with initial symptoms referable to the central nervous system (Kirby and McNaught, 1946; Eckhardt and Pilcher, 1950; Kaufman and Prieto, 1952; Krueger *et al.*, 1954;

Turner, 1954). In these instances however, some degree of pulmonary involvement can usually be detected. Malaise and low grade fever frequently accompany these various manifestations.

From the histologic standpoint, the characteristic lesion of nocardiosis appears to be granulomatous inflammation. These granulomas may contain giant cells of the Langhans type and closely simulate those produced by *M. tuberculosis* (Fig. III). However true caseation does not occur and there is a marked tendency for the granulomas to undergo suppuration (Fig. IV). It has been reported that such lesions in the lung have been mistaken for lipoid pneumonitis (Connar *et al.*, 1951). The etiologic agent can rarely be detected in hematoxylin and eosin stained preparations. However with Gram's and Ziehl-Neelsen stains it can usually be demonstrated, being irregularly gram positive and variably acid-fast.

Conclusive establishment of the diagnosis rests on recovery of the organism from lesions compatible with the disease. In addition it is desirable, whenever possible, to support this evidence by demonstration of the organism in pus and/or in tissue sections. As nocardia is part of the normal flora of soil, it may on occasion be encountered in sputum and in superficial lesions as an incidental finding (Mogabgab and Floyd, 1951). Further, the detection of acid-fast bacilli in direct sputum smears does not of necessity indicate infection with *M. tuberculosis* as acid-fast bacillary forms of nocardia species may be found in such preparations (Conant *et al.*, 1954). It is suggested that in all clinically diagnosed cases of tuberculosis having positive smears with repeatedly negative cultures nocardiosis be considered. Concentration procedures for the culture of *M. tuberculosis* will generally result in failure to recover *N. asteroides* (Benbow *et al.*, 1944; Conant *et al.*, 1954).

Therapy with sulfonamides, particularly sulfadiazine, has proved to be efficacious, although certain surgical procedures may be indicated in addition (Benbow *et al.*, 1944: Glover *et al.*, 1948; Jacobson and Cloward, 1948; Connar *et al.*, 1951; Strauss *et al.*, 1951; Conant, 1954; Conant *et al.*, 1954). However cases with widespread dissemination may be refractory and it is reported that cases with cerebral symptoms are seldom amenable to therapy. Administration of the drug should be continued for

several months after improvement has occurred (Connar *et al.*, 1951). The organisms isolated from our cases showed *in vitro* sensitivity to aureomycin and terramycin. Conant states that this is not unusual but that the organism *in vivo* does not appear to be sensitive to any of the known antibiotics (Conant, 1954). However, Krueger and associates (1954) have recently reported a case of brain abscess which recovered after surgical excision followed by aureomycin and penicillin therapy. Strauss and coworkers (1951) have shown that despite the *in vitro* sensistivity of various strains of nocardia to antibiotics only sulfadiazine gives satisfactory results in animal protection studies. Recently a report has appeared of a case with persistent nocardial infection of the skin and subcutaneous tissues of the foot, which was successfully treated with pregnenolone acetate (Lamb *et al.*, 1953). This drug, a steroid without toxic or metabolic effect, was selected because steroid hormones, including estrogen and testosterone, have been shown to have anti-fungal properties. The infection in this case was refractory to sulfonamide therapy.

## *Summary*

Two fatal cases of nocardiosis, occurring in residents of Central Texas, have been described. In both, the illness was chronic and constituted a diagnostic problem and in neither was nocardiosis considered during life. After autopsy the diagnosis was established by culture and by histologic studies.

The cultural characteristics of the strains of *Nocardia asteroides* isolated from these cases have been described.

As this disease in its earlier stages is amenable to therapy, it is suggested that nocardiosis should be borne in mind when cases of chronic unexplained infection are under consideration.

*Acknowledgment*

Photographs by R. M. Christopher and R. D. MacIntyre of the Medical Illustration Laboratory, Veterans Administration Center, Temple, Texas.

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# ANALYSIS OF BLOOD PRESSURES, HEART RATES, AND PULSE PRESSURES IN MEDICAL AND DENTAL STUDENTS*

FREDERICK E. EMERY

There is nothing new in the concept that heart and blood pressure are related and that each is governed somewhat by the other. Many reflexes induced by changing stimuli effect blood pressure and heart rate in opposite directions as Marey has postulated. This is not always the case and in exercise both usually go up. Buchanan found that the next diastolic period following commencement of exercise was shortened. Such delicate and rapid responses in heart rate and blood pressure to various stimuli may not always be evident. But in many well known reflexes, as the depressor reflex, carotid sinus reflex, venous reflexes, those concerned with special areas as spleen, pulmonary afferents, muscles, and skin, have added to our knowledge of heart rate and blood pressure. Thus it is no wonder that a set pattern for change in heart rate and blood pressure has not been established.

This report deals with blood pressures and heart rates in healthy adults with emphasis on coefficients of correlation and coefficients of variation.

*Methods.* A total of 952 medical and dental students were used and data for the coefficients of correlation were obtained after the subjects had adjusted to a steady state in the lying, sitting and standing positions. Readings were taken, also, immediately after mild and vigorous exercise. Mild exercise consisted of raising the body 18 inches to the top of a stool five times in fifteen seconds as recommended by Schneider. Severe exercise was running as long and as fast as could be tolerated and pulse rate and blood pressure taken immediately. Systolic pressure was taken with mercury manometers using the auscultatory method; diastolic pressure was taken as the level where a loud sound suddenly changed to muffled or lower sound. The students were not skilled in taking blood pressure but with supervision their readings soon checked fairly well with those obtained by the instructor.

## *Results*

A. *Systolic blood pressure.* The mean systolic blood pressure

* From the Department of Physiology and Pharmacology, University of Arkansas, Little Rock, Arkansas. Paper No. 1161 Journal Series, University of Arkansas.

lying, sitting and standing is 114 millimeters each (Table 1). After standing exercise systolic pressure is 122 mm. and a still further rise is noted after vigorous exercise. A study of the data shows that systolic pressure standing was higher than systolic pressure lying in 47 per cent of the subjects, lower in 45 per cent,

TABLE 1

*Mean Blood Pressure and Heart Rate in 952 Students*

| | Mean | Median | Standard Deviation | Coefficient of Variation | Probable Error |
|---|---|---|---|---|---|
| | | | SYSTOLIC PRESSURE | | |
| Lying | 114 | 114 | 8.99 | 7.88 | .1965 |
| Sitting | 114 | 114 | 9.04 | 7.93 | .2025 |
| Standing | 114 | 115 | 10.59 | 9.28 | .2313 |
| Mild Ex. | 122 | 122 | 12.59 | 10.32 | .2752 |
| Vigorous Ex. | 154 | 152 | 23.76 | 15.84 | .4857 |
| | | | DIASTOLIC PRESSURE | | |
| Lying | 74 | 74 | 8.98 | 12.13 | .1886 |
| Sitting | 77 | 77 | 8.30 | 10.65 | .1733 |
| Standing | 79 | 79 | 10.36 | 13.11 | .2213 |
| Mild Ex. | 81 | 80 | 11.03 | 13.61 | .2357 |
| Vigorous Ex. | 78 | 79 | 17.82 | 22.88 | .3812 |
| | | | PULSE PRESSURE | | |
| Lying | 40 | 40 | 9.20 | 23.00 | .2011 |
| Sitting | 38 | 38 | 8.81 | 23.18 | .1925 |
| Standing | 35 | 34 | 9.13 | 26.09 | .1996 |
| Mild Ex. | 41 | 40 | 11.46 | 27.97 | .2505 |
| Vigorous Ex. | 77 | 72 | 28.54 | 71.60 | .6205 |
| | | | HEART RATE | | |
| Lying | 72 | 71 | 9.46 | 13.14 | .2068 |
| Sitting | 79 | 79 | 9.76 | 12.20 | .2135 |
| Standing | 85 | 85 | 10.87 | 12.79 | .2396 |
| Mild Ex. | 96 | 95 | 13.00 | 13.54 | .2842 |
| Vigorous Ex. | 135 | 135 | 23.66 | 17.53 | .5167 |

and unchanged in 8 per cent. These figures are in line with Schneider and Truesdell who found that 56 per cent were higher, 26 per cent were lower and 18 per cent gave no change in systolic pressure from recumbency to standing. These systolic pressures are in normal range and therefore of value only in correlation studies as will be discussed.

B. *Diastolic pressure.* Diastolic pressure is considerably lower lying than standing; the figures being 74 and 79 mm. The

mean difference is 5 mm. which is about 10 times the probable error of the difference whereas a figure four times is considered significant.

Mild exercise raised the diastolic pressure very little. After vigorous exercise most diastolic readings were higher but some were much below normal so the mean of 78 mm. after vigorous exercise is nearly the same as standing (Table 1).

In a few cases auscultatory sounds could be heard all the way down to zero so that the diastolic pressure could not be determined. In these cases we have called the reading one millimeter so as to use it in our study. Dawson mentions readings of this type and Schneider and Lowsley report low diastolic pressures taken shortly after exercise.

When diastolic pressure standing is compared to diastolic pressure lying we find that 67 per cent are higher, 26 per cent are lower and 7 per cent are the same. These percentages are similar to those of Schneider and Truesdale who studied 2,000 men. They found that the diastolic pressure standing was higher in 79 per cent, lower in 6 per cent and unchanged in 15 per cent. Erlanger and Hooker in the early studies on diastolic pressure pointed out that diastolic pressure could be a little lower or higher when standing as compared with lying. Sewell found, in the normal subject, that pressures were higher sitting than standing. Since then abundant evidence supports a somewhat higher diastolic pressure standing.

C. *Pulse pressure.* The lowest mean pulse pressure was found on standing. Sitting, lying and exercise increase pulse pressure. The mean values (Table 1) are within normal ranges and need no further comment in this section. When pulse pressures standing are compared with lying 24 per cent are higher, 71 per cent lower and 5 per cent are the same.

D. *Heart rate.* One of the adjustments on going to the standing position from lying is an increase in heart rate. Schneider and Truesdell found, in 2,000 cases, only a few in which the rate was higher when lying. In the present study, standing heart rate was higher than lying in 95 per cent, lower in 2 per cent and the same in 3 per cent of the subjects. The increase on standing is 13 beats which is in line with others as reviewed by Dawson and Schneider.

E. *Schneider Index.* The range is from —5 to 18. The mean of 10.25 is somewhat above Schneider's findings but comparable to figures reported by Jackson for students.

F. *Coefficient of correlation.* One reason for this report is to obtain the coefficients of correlation referred to hereafter as (R). All comparisons possible on the data give a total of 210 (Rs). These are shown in table 2 and below each correlation is the probable error of each. This table is discussed beginning at the left and downward.

*Heart rates correlated with heart rates.* There are ten (Rs) in this group with values ranging from +0.815 when heart rates sitting were correlated with heart rates reclining to +0.190 when heart rates after vigorous exercise were correlated with heart rates reclining. All (Rs) are positive and all significant; *i.e.*, four or more times the probable error of the coefficient of correlation.

*Heart rate correlated with pulse pressure.* There are twenty-five (Rs) in this group of which fourteen are negative and eleven are positive. Four negative and one positive value are significantly correlated. These negative correlations mean that the slower the heart the greater the pulse pressure. This is a well known fact and therefore it is strange that more of these correlations are not significant. Schneider and Truesdell found the correlation between reclining and standing postural changes of pulse rate and pulse pressure was significant, the (R) being —0.290±0.014. Their figures for calculating (R) were the differences between lying and standing while the actual heart rate and pulse pressure were used for determining the (Rs) given in table 2. Therefore the correlation between heart rate and pulse pressure lying given in the table as +.008 ± .022 is not identical with Schneider's correlation.

*Heart rate correlated with diastolic pressure.* Twenty-two of the coefficients or correlation are positive and three negative. All of these negative correlations were with diastolic pressure after vigorous exercise. All (Rs) are small the largest being +0.202 when sitting heart rate is correlated with sitting diastolic pressure. Significant correlations occur nine times and are all found among the positive correlations. As a whole correlations between heart rate and diastolic pressure are small. Schneider and Trues-

TABLE 2

| | Heart Rate | | | | | Pulse Pressure | | | | | Diastolic Pressure | | | | | Systolic Pressure | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Vigorous Exercise | Standing Exercise | Standing | Sitting | Reclining | Vigorous Exercise | Standard Exercise | Standing | Sitting | Reclining | Vigorous Exercise | Standard Exercise | Standing | Sitting | Reclining | Vigorous Exercise | Standard Exercise | Standing | Sitting | Reclining |
| **Standard Exercise** | +.371*<br>.019 | | | | | | | | | | | | | | | | | | | |
| **Standing** | +.232*<br>.021 | +.684*<br>.012 | | | | | | | | | | | | | | | | | | |
| **Sitting** | +.243*<br>.021 | +.604*<br>.014 | +.722*<br>.009 | | | | | | | | | | | | | | | | | |
| **Reclining** | +.190*<br>.021 | +.580*<br>.014 | +.720*<br>.010 | +.815*<br>.007 | | | | | | | | | | | | | | | | |
| **Vigorous Exercise** | +.120*<br>.022 | −.005<br>.022 | −.095*<br>.022 | −.079<br>.022 | −.131*<br>.021 | | | | | | | | | | | | | | | |
| **Standard Exercise** | +.031<br>.022 | +.025<br>.022 | −.091*<br>.022 | −.078<br>.022 | −.008<br>.022 | +.560*<br>.015 | | | | | | | | | | | | | | |
| **Standing** | +.039<br>.022 | −.136*<br>.021 | −.069<br>.022 | −.069<br>.022 | +.006<br>.022 | +.311*<br>.020 | +.694*<br>.011 | | | | | | | | | | | | | |
| **Sitting** | +.037<br>.022 | −.062<br>.022 | −.086<br>.022 | −.021<br>.022 | +.019<br>.022 | +.350*<br>.019 | +.488*<br>.017 | +.673*<br>.012 | | | | | | | | | | | | |
| **Reclining** | +.022<br>.022 | +.002<br>.022 | +.006<br>.022 | −.003<br>.022 | +.008<br>.022 | +.355*<br>.019 | +.537*<br>.016 | +.745*<br>.010 | +.625*<br>.013 | | | | | | | | | | | |
| **Vigorous Exercise** | +.077<br>.022 | −.022<br>.022 | −.025<br>.022 | −.029<br>.022 | +.035<br>.022 | −.548*<br>.015 | −.308*<br>.020 | −.208*<br>.020 | −.228*<br>.021 | −.346*<br>.019 | | | | | | | | | | |
| **Standard Exercise** | +.066<br>.022 | +.012<br>.022 | +.080<br>.022 | +.063<br>.022 | +.079<br>.022 | −.135*<br>.021 | −.414*<br>.018 | −.250*<br>.020 | −.219*<br>.021 | −.244*<br>.021 | −.476*<br>.017 | | | | | | | | | |
| **Standing** | +.054<br>.022 | +.090*<br>.022 | +.093*<br>.022 | +.180*<br>.021 | +.103*<br>.022 | −.175*<br>.021 | −.242*<br>.021 | −.480*<br>.017 | −.197*<br>.021 | −.273*<br>.020 | +.389*<br>.019 | +.661*<br>.012 | | | | | | | | |
| **Sitting** | +.061<br>.022 | +.081<br>.022 | +.063<br>.022 | +.202*<br>.021 | +.098*<br>.022 | −.050<br>.022 | +.033<br>.022 | −.243*<br>.021 | −.552*<br>.015 | −.252*<br>.020 | +.355*<br>.019 | +.566*<br>.015 | +.591*<br>.014 | | | | | | | |
| **Reclining** | +.012<br>.022 | +.084<br>.022 | +.099*<br>.022 | +.114*<br>.022 | +.166*<br>.016 | −.119*<br>.022 | −.216*<br>.021 | −.342*<br>.019 | −.205*<br>.021 | −.606*<br>.014 | +.374*<br>.019 | +.544*<br>.015 | +.593*<br>.014 | +.657*<br>.010 | | | | | | |
| **Vigorous Exercise** | +.114*<br>.022 | −.011<br>.022 | −.083<br>.022 | −.071<br>.022 | +.088*<br>.020 | +.775*<br>.009 | +.292*<br>.020 | +.218*<br>.021 | +.175*<br>.021 | +.208*<br>.021 | +.092*<br>.022 | +.193*<br>.021 | +.195*<br>.021 | +.177*<br>.021 | +.148*<br>.021 | | | | | |
| **Standard Exercise** | +.107*<br>.022 | +.022<br>.022 | +.179*<br>.021 | +.020<br>.022 | +.064<br>.022 | +.269*<br>.020 | +.587*<br>.014 | +.397*<br>.018 | +.264*<br>.020 | +.282*<br>.020 | +.165*<br>.021 | +.524*<br>.016 | +.199*<br>.021 | +.391*<br>.019 | +.278*<br>.020 | +.437*<br>.018 | | | | |
| **Standing** | +.080<br>.022 | +.074<br>.022 | +.060<br>.022 | +.036<br>.022 | +.114*<br>.022 | +.215*<br>.021 | +.399*<br>.018 | +.579*<br>.015 | +.372*<br>.019 | +.331*<br>.019 | +.158*<br>.021 | +.395*<br>.018 | +.434*<br>.018 | +.348*<br>.019 | +.249*<br>.021 | +.360*<br>.019 | +.657*<br>.012 | | | |
| **Sitting** | +.077<br>.022 | +.042<br>.022 | +.012<br>.022 | +.020<br>.022 | +.076<br>.022 | +.202*<br>.021 | +.306*<br>.0198 | +.411*<br>.018 | +.566*<br>.015 | +.357*<br>.019 | +.139*<br>.021 | +.271*<br>.020 | +.278*<br>.020 | +.465*<br>.017 | +.276*<br>.020 | +.307*<br>.020 | +.546*<br>.015 | +.925*<br>.003 | | |
| **Reclining** | +.010<br>.022 | +.063<br>.022 | +.077<br>.022 | +.075<br>.022 | +.147*<br>.021 | +.238*<br>.020 | +.306*<br>.020 | +.348*<br>.019 | +.290*<br>.016 | +.464*<br>.017 | +.099*<br>.022 | +.377*<br>.019 | +.363*<br>.019 | +.462*<br>.017 | +.320*<br>.020 | +.330*<br>.019 | +.454*<br>.017 | +.608*<br>.014 | +.596*<br>.014 | |
| **Schneider's Index** | −.023<br>.022 | −.679*<br>.012 | −.753*<br>.009 | −.601*<br>.014 | −.598*<br>.014 | +.140*<br>.021 | +.005<br>.022 | +.112*<br>.022 | +.038<br>.022 | −.052<br>.022 | +.041<br>.022 | −.001<br>.022 | +.022<br>.022 | −.072<br>.022 | −.136*<br>.022 | +.075<br>.022 | +.066<br>.022 | +.072<br>.022 | −.015<br>.022 | −.216*<br>.021 |

The coefficients of correlation (R) are shown for all possible combinations. Beneath the "R" is the probable error of "R". If the "R" is four or more times its probable error the correlation is regarded as significant and marked by an asterisk.

Heart Rate — Pulse Pressure — Diastolic Pressure — Systolic Pressure

dell found the coefficient of correlation with heart rate and diastolic pressure was 0.245 ± 0.016. This correlation as judged by their discussion is positive but it is not so indicated.

*Heart rate correlated with systolic pressure.* There are twenty-two positive and three negative (Rs). All are small and six of the positive ones show significant correlation. Schneider and Truesdell studied the pressure changes in pulse rate and systolic pressure "for the recumbent to standing posture" for their 2000 cases and found the coefficient of correlation only —0.213 ± 0.014. This is in line with the present data in that the (R) is small but not in agreement in that the correlation in their study was negative. In the present study all three negative correlations were in the vigorous exercise group where changes from standing norms were great. Thus the concept of Marey's law is best exhibited when changes in blood pressure are extreme. This was brought out by Wickwire with cats in which blood pressure was low and heart rate up as the result of hemorrhage. A reduced heart rate is a common occurrence in laboratory experiments dealing with a sudden increase in blood pressure as found after injections of adrenalin.

*Heart rate correlated with Schneider's test.* High correlations were found, these range from —0.023 after vigorous exercise to —0.753 after standing. All are significant except heart rate after vigorous exercise. Why all correlations are negative is not clear but fits into the known physiological fact that fast hearts are associated with poor efficiency which Schneider's test is designed to measure.

*Pulse pressure correlated with pulse pressure.* Table 2 there are ten correlations possible. All are positive and all significant. They range from +0.311 to +0.745 which are among the highest in table 2. Since they are all quite significant one is led to think that pulse pressure may be of more importance than the usual emphasis put upon it. Erlanger and Hooker considered a small pressure as a factor in albuminuria and McCrea and Wiggers have proof that pulse pressure variations dominate the production of reflex cardiac changes. A similar mechanism is suggested by Roth and Kirsch for auricular irregularity.

*Pulse pressure correlated with diastolic pressure.* Twenty-four of the (Rs) are negative and in 23 of these correlations are sig-

nificant. The one positive correlation is with pulse after standard exercise compared with diastolic sitting. The (R) is +.033 a figure too small to have any meaning. Thus when the diastolic pressure is high, pulse pressure would be less, unless systolic pressure is proportionally elevated, and a negative correlation between pulse pressure and diastolic pressure would result. Mean figures do not give evidence of individual changes as well as figures for (R) but, as seen in table 1, mean values show that diastolic pressure is more sensitive to posture changes than is systolic pressure. Schneider and Truesdell found the correlation for pulse pressure and diastolic pressure to be very high; in mild exercise being —0.980 ± 0.014.

*Pulse pressure correlated with systolic pressure.* All 25 (Rs) are positive and all are significant. They range from 0.175 when pulse pressure sitting is correlated with systolic pressure after vigorous exercise to 0.775 when they are both correlated after vigorous exercise. All the correlations in this group are as a whole larger than those just discussed for diastolic pressure and pulse pressure. They also differ in that in this group all correlations are positive while 24 of the correlations with pulse pressure and diastolic pressure are negative. Schneider and Truesdell found (R) to be +0.773 ± 0.006 between pulse and systolic pressures.

*Pulse pressure correlated with Schneider's test.* Since pulse pressure is not directly associated with Schneider's test there is very little reason for this correlation except to complete the discussion on pulse pressure. Four of the five (Rs) are positive and all are small. However, two are significantly correlated with Schneider's test but the amount of correlation is not impressive (Table 2).

*Diastolic pressure correlated with diastolic pressure.* The (Rs) total 10 and nine are positive. All are significant, rather large and range from 0±355 to +0.661. The negative one is —0.476 and occurred when diastolic pressure after vigorous exercise is correlated with diastolic pressure after standing exercise.

*Diastolic pressure correlated with systolic pressure.* All 25 (Rs) are positive and all significant. This clearly shows that systolic pressure is associated with changes in diastolic pressure. Even after vigorous exercise where low diastolic pressure may be present the (R) is small (+0.092) but significant. From this

the (Rs) range up to 0.465 found with diastolic pressure, sitting, is correlated with systolic pressure, sitting. Schneider and Truesdell found the (R) with systolic and diastolic pressure was +0.219 based on postural changes from recumbency to standing.

*Diastolic pressure correlated with Schneider's test.* Here again as with pulse pressure there is no reason to consider correlation with Schneider's Index except to complete the discussion on diastolic pressure. Three of the 5 correlations are negative and all are small the (R) with diastolic pressure lying and Schneider's test is the largest being —0.136 and the only significant one in this group.

*Systolic pressure correlated with systolic pressure.* All ten correlations are positive and all significant. The (Rs) range from +.307 to +.925 showing that systolic pressure in an individual tends to hold its relative position to the mean and is changed only slightly by posture and exercise.

*Systolic pressure compared with Schneider's Index.* Since systolic pressure is one of the factors composing Schneider's test it would seem to be definitely related and correlated with the test. Table 2 does not bear this out. In fact the largest and only significant (R) in this group is —0.216 obtained in the reclining position. The smallest is —.015 obtained in the sitting position. The three remaining (Rs) are negative and small.

*Discussion.* Mean systolic pressures lying, sitting and standing are the same, all being 114 mm. (Table 1). These means values are incidental and not in agreement with the usual concept that standing increases systolic pressure. They are in agreement with the data of Schneider and Truesdell in that systolic pressure is higher standing in about one-half of the subjects as pointed out under results.

Diastolic pressure is higher standing than lying and the mean difference of these divided by the probable error of the difference is more than ten where as a figure of four or more by this method of analysis is considered significant.

Pulse pressure is 5 mm. higher lying than standing and this mean divided by their probable error of the difference is significant, being 10.

Heart rate is noticeably higher standing and the mean dif-

ference between standing and lying is 13 beats or more than 30 times their probable error of the difference.

Coefficients of correlation are not common in studies on blood pressure. The paper of Schneider and Truesdell was the only one found. Burlage ran correlations for age and blood pressures using data from 4,972 girls taken from subjects reported by Alvarez. The (R) was —.052 and P.E. of (R) ±.010 figures which would indicate the correlation with age was negative and scarcely significant. Burlage considers the (R) not to be significant though it is five times its probable error.

Table 2 shows 210 coefficients of correlation. There are 154 positive and 95 are significant, 56 are negative and 32 are significant as indicated by an asterick when the (R) is more than four times the probable error of (R).

These positive correlations are found throughout the table and rather large figures are obtained when heart rates are correlated with heart rates in various positions and after exercise. The same is true of pulse pressure correlated with pulse pressure, diastolic pressure with diastolic pressure and systolic pressure with systolic pressure. But when correlations are with unlike readings the (Rs) are usually not so large. For example systolic pressure correlated with heart rate give (Rs) that are all small and nearly all without significance. However, pulse pressures correlated with diastolic pressures give negative correlations and the figures are rather large.

It is surprising to see Schneider's test correlating so well with the other data. Eight of these twenty (Rs) or 40 per cent of the total are significant as compared to all correlations where 127 of the 210 (Rs) or 60 per cent are significant. Heart rate is more closely correlated with Schneider's Index than systolic pressures, diastolic pressures or pulse pressures (Table 2).

Mean values tell little about comparative variations or degree of scatter of the variates composing the means. Also the size of the variates influence not only the means but the deviations from the mean so that comparisons between means should be brought to a common expression. This Pearson calls the coefficient of variation.

These coefficients are included in table 1. It is interesting that systolic pressure is least variable, it has the smallest coefficients

of variation. Systolic pressure is followed by heart rate, diastolic and pulse pressures respectively.

*Summary*

A study of 952 students using heart rate, pulse pressure, diastolic pressure, systolic pressure and Schneider's test have been made. Mean, median, standard deviation, coefficient of variation, probable error and coefficient of correlation have been made in the lying, sitting and standing position and after mild and vigorous exercise. A total of 210 coefficients of correlation have been studied. There are 154 positive correlations with 95 significant and 56 negative correlations with 32 significant. Correlations are highest when like reading are correlated in different positions. Heart rate correlated with heart rate, pulse pressure with pulse pressure, diastolic pressure with diastolic pressure, and systolic pressure with systolic pressure gives a total of 40 correlations and all are significant.

A study of coefficients of variations, reveals that systolic blood pressure is least variable followed by heart rate, diastolic pressure and pulse pressure respectively. The mean Schneider's Index is 10:25.

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# THE FACTORIAL STRUCTURE OF THE RORSCHACH TEST*

AUSTIN FOSTER

Since its inception in 1921, the Rorschach test has been the subject of more investigations than any other clinical testing instrument except the Binet-Simon intelligence test and its modifications. A comprehensive bibliography (Klopfer and Kelley, 1946) reveals 642 books and articles written prior to September, 1945, which dealt with the test. This mass of literature reflects, if nothing else, the tremendous interest engendered by Rorschach's experiment.

Academic psychology ignored Rorschach's work at first. This is understandable, since the "form-interpretation experiment" was developed in a psychiatric milieu steeped in the theories of Freud and Bleuler. There was, at this time, little intercourse between academic psychology, dominated by the Wundtian interest in the "normal, generalized human mind," and early "dynamic" psychiatry, represented in Switzerland by Jung and Bleuler.

Later, as academic psychology turned more and more to the investigation of "individual differences" and the problems of motivation and adjustment, there came a gradual assimilation of the theories and techniques of psychiatry, and the first significant psychological interest in the Rorschach test developed.

At first, the test had more detractors than enthusiasts, and only those psychologists who worked in the borderland between psychology and psychiatry made serious attempts to become expert in the use of the test. Most psychologists had only contempt for a technique which had almost as many methods of "scoring" as users, which depended upon the use of "intuition," and which, allegedly, demanded a deep insight into personality not available to many psychologists. The orientation of the developers of the test was such that a literature developed almost devoid of references to reliability and validity except for bland

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assurances that the test *was* valid. Occasionally, a published comparison of a blind Rorschach analysis of a single case with the psychiatric data was offered as "proof" of the validity of the method.

Nonetheless, evidence gradually accumulated that the test, in the hands of a careful examiner, did yield valid measures of certain aspects of the personality (Benjamin and Ebaugh, 1938). Thus, the test demonstrated sufficient "global" validity to insure attention and to inspire further investigation. Here, however, a difficult problem arose: validity in the sense of the utility of the method had been demonstrated, but there were nowhere satisfactory statements of the operations by which the expert arrived at his interpretations. Many Rorschach workers insisted that it was impossible to analyze their interpretational methods in a way compatible with strict scientific procedure: since the significance of each score depended upon the "total configuration," analytic methods would destroy that which they sought to measure.

Although writers on the Rorschach test are reasonable enough in insisting that the method is not reducible to psychometric scores, one suspects that their attitude has been, at least in part, defensive, since experimental investigation has frequently failed to demonstrate relations claimed by workers with the test. For example, whole responses, indicating intelligence and "organizing and integrating ability" according to theory, have failed to correlate with intelligence tests, the typical correlation being of the same order as the 0.13 obtained in the present study between *W* and the Wechsler vocabulary test. The Air Force found the test valueless in pilot selection (Guilford, 1947).

As Rapaport (1946 )says: "In its history, the Rorschach Test has undergone a variety of vicissitudes. Extensive revisions of its scoring were introduced, new methods of group administration were developed, a 'multiple choice' variation was introduced, parallel sets of blots were developed. None of these, however, has solved the formidable problem in the application of the Rorschach Test: its standardization and validation . . . In spite of its great impact, the Rorschach Test has failed to fulfill the job of breaking through the wall of statistical security behind which the I. Q. intelligence testers and the champions of per-

sonality questionnaires withdrew to defend themselves against the entrance of dynamic psychological thinking into clinical psychology."

In Hertz's (1941) detailed review of Rorschach literature over twenty years, the most noticeable aspect is the lack of validation studies of a type which would be acceptable to the psychometrician.

There are a number of meanings given the term "validity" in reference to projective techniques. Many Rorschach studies have demonstrated an impressive *global validity*, *i.e.*, demonstration of the utility of the method by making correct predictions from the test results. Global validity, however, does not involve any statement of the operations by which the results were obtained.

*Differential signs*, *i.e.*, differences in *scores* of one kind or another, are satisfactory to the psychometrist if they are based upon adequate sampling. This method has been used to determine the characteristics differentiating one pattern of adjustment from another, or one disease entity from another with considerable success. However, most studies in this field have been fragmentary, in that they dealt with signs which distinguished one condition from another, when a rigorous development demands signs which differentiate one condition from *all others*. Rapaport criticizes studies dealing with single groups as follows:

> "Such studies may find indicators which distinguish their experimental group from a group of normal cases, but they cannot make clear to what extent those indicators would distinguish any or all other clinical groups from the 'normal' population. Furthermore, they do not meet the problem of distinguishing one clinical group from another, a problem always in the foreground of clinical psychological practice. The characteristic patterns of a single nosological group thus discovered cannot be used as diagnostic indicators; they may or may not be necessary parts of the diagnostic picture of the group in question, but are generally far from sufficiently describing it and differentiating it from all other nosological groups. Nor do studies which lump together all kinds of 'neurotics' or 'depressions' or 'schizophrenias'—useful as they may be—attempt to cope with the problem of validation of the test."

Thus, we see that the development of adequate differential signs necessitates the systematic investigation of all clinical groups and the many "normal" patterns to be fully satisfactory. This goal is almost impossible of achievement. Beck has kept a careful record of each Rorschach protocol which he has in-

terpreted for many years, and he may eventually accumulate a sample adequate in all respects. At present, the best study of this type is that of Rapaport. His investigation covers a wide range of clinical material and involves carefully defined nosological groups. However, even this study has serious defects: the organic disorders are omitted, as are the character neuroses and the various alcoholic and psychopathic personality syndromes. The normal population is represented by a highly restricted sample which is far from representative of the general population. Furthermore, no attention is given to age differences. Since Rapaport's clinical sample is highly biased in favor of the economically and intellectually superior, there is considerable doubt that his results are applicable to the lower half of the intelligence distribution. Certain clinical groups are represented by samples of less than ten cases. In spite of these objections, however, Rapaport's work represents a significant contribution to the development of adequate differential signs.

As we have seen, satisfactory differential signs can be achieved only with the greatest difficulty. In addition, there is a further objection to their use as the principal method of demonstrating validity: the signs are entirely empirical. The laborious collection of differential signs is inherently a less elegent procedure than the development of a theoretical rationale which accounts for the observed signs and allows prediction of the Rorschach pattern from a knowledge of the psychological processes operating in a subject at the time of the testing. *Process* validations, *i.e.*, demonstration of the psychological processes giving rise to variations in scores, is the ultimate aim in establishing the valdity of the Rorschach test.

Unfortunately, the development of a theoretical rationale has been very slow. Rorschach frankly admitted the purely empirical nature of his results. The basic sources offer only fragmentary material, much of it based upon crudely analogical reasoning. For example, Klopfer, writing of *K* and *k* responses as anxiety signs, offers the following explanation: "Plain diffusion responses (K) and toned-down shading effects (k) invariably indicate insecurity and anxiety of the free-floating type. The physical qualities of these shading effects seem to reflect the corresponding qualities of the inner life of the subject:

haziness and fogginess, created by free-floating anxiety. K and k seem to indicate a flight from the more sensuous shading effects, whenever these effects arouse so much anxiety and guilt that the subject is not able to accept them. For this reason, the diffusion responses often have a dysphoric tinge."

In contrast to the casual, analogical explanations, of which the quotation above is characteristic, Rapaport has attempted to sketch in the psychological processes underlying the Rorschach test in terms acceptable to general psychology. His work has appeared too recently to allow an adequate testing of the rationale developed, but, at the very least, it initiates a new and mature phase in the difficult work of developing a set of constructs adequate for the description of personality.

Global validity demands only matching techniques for its demonstration, *i.e.*, statements such as "86 per cent agreement was found between blind Rorschach diagnoses and psychiatric diagnoses." The approach through differential signs leads to tests of the significance of differences such as "t" and "Chi-square." Process validation, however, implies continuity and leads to correlational techniques. Thus, it becomes necessary to examine the nature of Rorschach scores and the propriety of their quasi-mathematical treatment. If, as Pratt (1939) maintains, all science can be reduced to correlation, we should expect any Rorschach rationale to be stated ultimately in terms of correlations, yet, as we have seen, it is at just this point that the demonstration of the validity of the test has failed.

The typical psychometric instrument offers a series of tasks in which the responses are forced into a highly restricted number of categories. Thus, a response is scored "right" or "wrong" or, in the extreme case, the response receives positive or negative weights of varying magnitudes. In any case, the subject's response is highly structured and stereotyped.

In a projective test, such as the Rorschach, the subject is given the greatest possible freedom in dealing with the test material. Thus, the subject may deal with a certain part of the material or not, as he prefers. Clinicians have found that a psychological variable such as anxiety may find expression in a number of equivalent ways. The adoption of a preferred way of expressing anxiety on the test frequently leads to the avoidance of alternate

possibilities. If we assume on clinical grounds that anxiety may be expressed by (a) an increase in *dd*, *de*, and *s* responses, (b) an increase in crude shading responses, (c) an increase in vague *W* responses, (d) constriction, with no determinants other than form and reduced *R*, or (e) color shock and impaired form level on chromatic cards, then it is apparent that no one of these measures would yield more than minimal correlation with a criterion, nor would the determinants correlate appreciably with each other. An obvious solution of the difficulty is the weighted summation of the indicators; this has rarely been attempted for two reasons: with few exceptions, skilled Rorschach workers have been unskilled in psychometric procedures and not infrequently have been hostile toward their use; similarly, few competent psychometrists have had sufficient acquaintance with Rorschach interpretation to deal with Rorschach scores in any but the most mechanical fashion.

A second source of difficulty is found in the assumption of linearity which underlies almost all statistical techniques utilized in psychological research. In the Rorschach, *R*, the number of responses, may vary through a range limited at one end by zero and by the patience of the examiner at the other. Few, if any, of the Rorschach scores are monotonic functions of the number of responses, nor are they independent of *R*. In samples in which *R* varies between wide limits, linearity cannot be legitimately assumed.

A third source of difficulty lies in the effect of grouping uncorrelated or negatively correlated variables together under a single scoring symbol. This state of affairs, shocking to psychometrists, arises naturally out of the use Rorschach workers make of their "scores." As Rapaport notes: ". . . scoring categories are merely aids to facilitate an appraisal of what has happened in the course of the test, and . . . a 'correct' set of scores is not an end in itself." In other words, the clinician uses "scores" merely as pegs to aid him in keeping in mind a number of variables. In his interpretative work, he goes far beyond the rather crude differentations represented by the scoring scheme. Thus, from clinical experience, we have the propositions that integrated, definitive whole responses are a function of intelligence and organizing ability, while vague, amorphous whole responses

such as "it looks cloudy" or "mud" arise in part from the pressure of anxiety. Again, absurd, arbitrary whole responses bearing no relationship to the formal characteristics of the plates come about through the development of a psychosis. The Rorschach analyst sees no objection to grouping all these as *W* responses, since he then proceeds to a qualitative breakdown, but it would be difficult indeed to regard *W* as a homogeneous psychometric score.

It seems essential that Rorschach symbols be markedly refined before they are used as the basis for a psychometric study. This, of course, is not incompatible with the clinician's use of the cruder scores in routine practice. Zubin (1941, 1943) has proposed a scheme for converting clinical scoring into univocal psychometric scoring, and Rapaport, in the work cited above, has adopted a number of scoring refinements for statistical purpose which he does not use in daily clinical practice.

The Rorschach shares a fourth source of difficulty with all measures purporting to deal with some area of personality: the difficulty in finding adequate criterion measures. In spite of the incomplete validation of the Rorschach test, we frequently see it used as if it were a fully confirmed instrument, due to the lack of other satisfactory criterion measures. The only resolution of this difficulty is simply to do the best that one can, working carefully to insure maximum validity in whatever criteria are available. The present situation in personality theory is no whit different from that encountered in intelligence testing: teachers' rating were used to validate the Binet, which was then used to show that teachers' ratings were inaccurate.

The argument is here advanced that, with careful control of the sources of difficulty outlined above, the Rorschach test may be treated as a psychometric instrument as well as a clinical device, and that psychometric investigation is essential for the confirmation and clarification of the principles of interpretation developed in practical work with the test. The interpretations of Rorschach scores are constructs, and a logical construct is defined by its correlations. If a construct is to have scientific value, it must be defined by two or more independent measurements. The value of the Rorschach test, then, may be defined in terms of the psychological significance of its correlates: it is a successful

test to the extent that it affords an economical index to psychological traits (constructs) of general interest in the fields of personality and psychopathology.

Within the framework of correlational analysis, the most potent tool for reducing a multiplicity of correlations to a parsimonious set of basic constructs is multiple factorial analysis. Of the varied (although mathematically equivalent) methods developed, Thurstone's method of rotated centroid analysis (Thurstone, 1947) has proven most valuable for psychological investigation (Wolfe, 1940).

The present investigation is an attempt to arrive at a valid description of the processes underlying certain Rorschach scores by determining their correlation with measures of certain constructs in the field of personality, these measures involving operations of a radically different sort from those involved in the Rorschach test. This study is, apparently, the first attempt to relate the Rorschach test to other factorial studies in the field of personality. Thurstone (1944) included certain Rorschach variables in his study of perception, isolating a doublet factor which was not further investigated; however, he purposely excluded all of the usual measures of personality traits from his battery. Hsu (1947) recently analyzed the content of responses to Card I of the Rorschach test with the aim of discovering the number of factors necessary to account for the obtained correlations; however, his approach did not deal at all with scores normally utilized in interpreting the test.

*The Experimental Variables.* As mentioned previously, the difficulty in finding adequate criterion measures in the field of personality creates a serious problem. To isolate factors underlying the Rorschach scores, one must obtain a matrix which includes at least two other measures of whatever factor there may be. Three principal types of measures are available to the psychologist: rating scales, tests, and questionnaires. There are serious objections to each device. The rating scale is notoriously unreliable, even under optimum conditions. Correlations so derived are minimal, since reliability coefficients are nearly always under 0.60, and validity coefficients, when obtainable, are usually below 0.50. Tests, particularly those based directly upon absolute or interactive measurements, are superior technically, but are

almost non-existent except in the areas of human personality labeled "intelligence" and "abilities." The third category, questionnaires, are predominately of a type open to serious objection: for the most part, they are composed of items selected because their content appeared to be relevant to a conception under investigation by the creator of the "test." Thus, such a question as "Are you afraid of high places?" would be considered an index to phobic disturbances. The examiner assumes that the question means the same thing to the subject that it does to himself, allowing no place in his thinking for the existence of idiosyncratic perception; he also assumes that the subject is ready and able to respond truthfully. Neither of these assumptions is entirely justified.

Cattell (1946) has been particularly critical of the use of personality questionnaires, terming the questionnaire "the nadir of scientific inventiveness." However, even Cattell admits the necessity for utilizing questionnaires for research purposes, since other measures are frequently unavailable at the present time.

Many of the objections to the use of personality questionnaires can be overcome by refusing to adopt *a priori* item classification based upon superficial content. When a measure labeled "*introversion*" and one labeled "*neuroticism*" correlate 0.95, two assumptions are possible: one, that the two variables are, in fact, identical; the other, that one or both labels are incorrect. Since in this case we have a test doublet, it is impossible to decide upon a proper designation with any assurance. The proper procedure here is to explore the further correlations of the two devices in order to arrive at a logical construct with the greatest reductive power and logical consistency. If the two measures also correlate with scales derived in different fashion, *i.e.*, behavioral observations or objective test data, then there is considerable assurance that one is dealing with a variable of psychological significance, not with a trivial product of item similarity.

Ideally, tests of this type should be derived empirically on the basis of item correlations (particularly on the basis of factorial studies), which are labelled only after their further correlations have been determined. Another method of test construction of some interest is the development of empirical scales differenti-

ating groups. The *Strong Vocational Interest Blank* utilizes this principle. In personality measurement, the Minnesota Multiphasic Personality Inventory is the only questionnaire based upon this method. In this case, items discriminating between various clinical syndromes and a population of normals were used to create the scale. Unfortunately, the method was not extended to deal with the problem of selecting items which differentiated a clinical syndrome from both normals of all types *and all other clinical entities*. Hence, the test suffers from extremely high intercorrelations between variables which differ greatly in their definition and prognosis (Hathaway and McKinley, 1943).

Test users unanimously agree that questionnaire type measures are easily faked, both deliberately and "unconsciously." As Cattell points out in the volume cited previously, this limits the effective use of questionnaires to *research*, in which the faking factor can be fairly well controlled. The indiscriminate use of these inventories in employee selection and other situations in which the subject has a strong interest in the outcome of the test is, to say the least, ill-advised.

These, then, were considerations which influenced the choice of personality measures included in the present study. All three types of measures were utilized, but minimal use was made of *a priori* questionnaire.

### *Correlation Matrix I*

Data from Rapaport's intensive study of a group of rather rigidly defined "normals" were selected for analysis. Three considerations determined this choice: first, this is the most thoroughly explored and reported collection of clinical data from normal subjects available; second, the factorial investigation of these data would allow for a critical evaluation of the theoretical constructs developed by Rapaport and his co-workers, which have greatly influenced the field of clinical psychology; and third, the Rorschach data are such that the difficulties outlined in Chapter I above are minimized. In regard to the third point, the *R* in Rapaport's group varies only within narrow limits, thus reducing one source of difficulty; the test scores are refined for statistical purpose, thus eliminating another source.

Rapaport's group consists of 54 randomly-selected members of the Kansas Highway Patrol. They are predominately of a rural background, and, with few exceptions, possess a high school education. Most of the group fall in Wechsler's bright normal classification, with a mean I.Q. of 116. Full infor-

mation on the characteristics and selection of the group are available in the work cited.

The personality measures available for this group include the Wechsler-Bellevue Adult Intelligence Scale, the brief form of the Babcock Test for Mental Efficiency, a sorting test, a qualitatively reported version of the Hanfmann-Kasanin Concept-Formation Test, Orbison's Word Association Test, various clinical ratings, and a detailed presentation of the Rorschach data.

Of these data, the Wechsler-Bellevue vocabulary test was selected for inclusion in the factorial study for two reasons: first, the vocabulary test is one of the best measures of verbal intelligence, which is assumed to be one of the factors reflected in Rorschach test scores; second, it affords an opportunity to appraise the personality correlates of intelligence. The Wechsler-Bellevue verbal scale I.Q. was included for the same reasons.

The psychiatrist's rating of anxiety was included, since the Rorschach purports to provide an index to a subject's patterns of anxiety. Other clinical ratings which would have been useful were omitted because the distributions of the ratings were poorly suited for correlational techniques.

The Rorschach variables available for inclusion in the battery were limited by the necessity for avoiding excessive "experimental dependence." Thus to include both *M* (human movement) and *H* (human content) scores in the same matrix would introduce a great deal of specific variance into the common factor space and distort the results. The following were finally selected:

1. *R:* the number of responses.
2. *W:* the number of whole responses.
3. *M:* the number of responses involving human movement.
4. *Sum C:* the weighted summation of color responses.
5. "Special *F* plus: the number of highly articulated responses.
6. *F*+%: the per cent of accurately seen responses.
7. *Fv:* the number of vague, indeterminate concepts used.
8. *Sum Shading:* the total number of shading responses.

The correlational matrix was obtained by means of tetrachoric correlations, using the Cheshire tables. In the event that a variable was divided into more than two categories, the score nearest the median was selected to dichotimize the measure, in order to insure maximum validity for the resulting correlations. Table 1 presents the obtained intercorrelations.

### *Correlation Matrix II*

A second matrix was desired for a number of reasons. It is a very desirable check upon the psychological meaningfulness of any factor obtained; "residual" factors frequently appear which are purely mathematical artifacts without psychological significance: these will not normally reappear in a different matrix. It also provides a test of the results obtained from the rotation of axes in the factor space: frequently alternate axis locations are available which meet the criteria for simple structure and lend themselves to psychological interpretation; this indeterminancy may be resolved by factoring a second matrix when Cattell's principle of proportional profiles is available as a test

of the stability of the structure obtained. Factor loading, of course, will differ as a function of the range of talent, but the essential structure should either be identical or reflect real difference in the two populations. For these reasons, the Rorschach test and a number of other measures were given to the group described later in this chapter.

In order to adhere as closely as possible to the plan developed in Chapter I for the use of the Rorschach as a psychometric instrument, a major modification was introduced: each subject was instructed to give at least three responses to each card (if he had given fewer) through the first nine cards of the test; on card ten, which is normally much more productive, each subject was urged to give at least six responses. No limitation was placed upon the number of responses given, but only the 33 responses called for as a minimum by the scheme given above were scored. Thus, each person's scores were based upon a constant "N" of 33.

For the second matrix, it was desired to use data obtained from a markedly different segment of the population; fulfilling this requirement, college students, who of course differ both in intelligence and cultural background from the patrol, were used as subjects. No attempt was made to obtain an unbiased sample of the population since factorial methods are concerned primarily with the identification of factors rather than with normative problems. Of greater importance was the problem of obtaining a group varying widely in adjustment whose test attitude would be one which would minimize faking so that questionnaires could be included in the battery with confidence. Accordingly, subjects were taken from students at The University of Texas who volunteered in response to an announcement that interested students could take a battery of tests, including the Rorschach, and receive an interpretation of the results. Psychology majors and others likely to have some knowledge of the tests were excluded. The finally selected group of 28 students were administered the following tests:

1. The Allport-Vernon Scale of Values.
2. The Minnesota Multiphasic Personality Inventory.
3. The Bernreuter Personality Inventory.
4. The Wechsler-Bellevue Adult Intelligence Scale.

The scale of values was included in order to investigate the relationships existing between interests and other facets of personality; four of Allport's six categories were used: theoretical, economic, aesthetic, and political; social and religious scales were omitted since they do not appear to be well-defined or consistent scales.

The Minnesota Multiphasic was included since its standardization has been somewhat more rigorous than that of most questionnaires. The *Mf* scale was omitted since the present group included both sexes. *Hy* (hysteria) and *Hs* (hypochondriasis) scales were discarded due to the limited variance in this group, which attenuated the resulting correlations severely.

Since the four original scales of the Bernreuter Personality Inventory have been shown to be reducible to (and predictable from) the two Flanagan scales based upon a principle components analysis, only the *F-IC* and *F-2S* variables were used.

The Wechsler-Bellevue vocabulary test was again included; however, the "VIQ" reported in the second matrix is not Wechsler's verbal scale intelligence quotient, but the average of the "essentially verbal" subtests: information, comprehension, and similarities. These are shown by Rapaport to be relatively stable and free from impairment by maladjustment. Another hypothesis by Rapaport led to the inclusion of a third variable dealing with Wechsler-Bellevue scores: digit-span scatter (DSS). Rapaport and other clinicians assume that scores on the digit-span subtest markedly lower than those of the stable verbal tests usually indicate impairment of attention as a function of anxiety. Hence, a measure of the discrepancy between digit-span and the "essentially verbal" tests was included.

Since *M* and *Sum C*, the component parts of Rorschach's "Erlebnistyp," were included in the first battery, it was decided to use the *M: Sum C* ratio in the second matrix in order to investigate the performance of the more complex variable, which is basic in Rorschach literature. Whole responses were divided into two groups: first, vague wholes (Wv), responses in which the entire blot was used as a gross unit, and second, ordinary wholes (Wo), responses in which at least a minimum degree of articulation was present. Finally, Klopfer's *FM* (animal movement) score was included in the hope of gaining some information regarding the disputed relationship between animal and human movement responses.

Each questionnaire-type test was machine scored, using standard IBM equipment. Each Rorschach record was scored twice: once by the author and independently by an advanced Rorschach student. The occasional disagreements in scoring were submitted to a third Rorschach analyst.

In computing the inter-relationship of these variables, product-moment correlations were used exclusively. The resulting correlation matrix is shown in Table 3.

### *The Factorial Results*

The tables of intercorrelations were factored by Thurstone's (1947) complete centroid method. Factorization was continued until not more than one residual greater than 0.20 remained. Factors were rotated by pairs, plotting each pair graphically, but performing the indicated rotations algebraically for the sake of accuracy. The first matrix yielded four factors, with no residual as large as 0.20; the second matrix required five factors to account for the correlations, with one residual greater than 0.20. Since no convincing evidence for oblique axes appeared in the course of the transformation of the values, the final factors reported are orthogonal.

The four factors of the first matrix were rotated twelve times, although adequate simple structure appeared earlier. The centroid and rotated factor loading are presented in table 3.

TABLE 1

*Correlation Matrix, Group I*

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| R ..... 1 | | +.12 | +.60 | +.37 | +.25 | +.49 | −.07 | −.20 | +.11 | +.60 | +.47 |
| M ..... 2 | +.12 | | +.02 | +.45 | +.38 | +.18 | +.71 | +.38 | −.67 | .00 | −.18 |
| Sum C ..... 3 | +.60 | +.02 | | −.08 | .00 | +.64 | −.17 | −.42 | +.33 | +.53 | +.58 |
| Sp F+ ..... 4 | +.37 | +.45 | −.08 | | .00 | +.11 | +.68 | −.19 | −.72 | −.18 | −.07 |
| Voc ..... 5 | +.25 | +.38 | .00 | .00 | | +.13 | .00 | +.55 | −.09 | +.12 | −.43 |
| W ..... 6 | +.49 | +.18 | +.64 | +.11 | +.13 | | −.21 | −.46 | +.25 | +.20 | +.26 |
| F+ Per cent ..... 7 | −.07 | +.71 | −.17 | +.68 | .00 | −.21 | | +.18 | −.82 | −.19 | +.09 |
| Verb IQ ..... 8 | −.20 | +.38 | −.42 | −.19 | +.55 | −.46 | +.18 | | −.21 | −.18 | −.42 |
| Fv ..... 9 | −.11 | −.67 | −.33 | −.72 | −.09 | +.25 | −.82 | −.21 | | +.53 | +.15 |
| Sum Shading ..... 10 | +.60 | .00 | +.53 | −.18 | +.12 | +.20 | −.19 | −.18 | +.53 | | +.42 |
| Anxiety ..... 11 | +.47 | −.18 | +.58 | −.07 | −.43 | +.26 | +.09 | −.42 | +.15 | +.42 | |

TABLE 2

| | | FACTOR MATRIX I (before rotation) | | | | | FACTOR MATRIX I (after rotation) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | I | II | III | IV | $h^2$ | I | II | III | IV | $h^2$ |
| R ..... | 1 | +.73 | +.32 | +.24 | +.19 | .73 | +.71 | +.28 | +.34 | +.16 | .72 |
| M ..... | 2 | +.54 | −.62 | +.27 | −.18 | .78 | +.14 | −.45 | +.75 | .00 | .78 |
| Sum C ..... | 3 | +.59 | +.60 | +.16 | −.07 | .74 | +.69 | +.50 | .00 | +.12 | .74 |
| Sp F+ ..... | 4 | +.59 | −.47 | −.34 | +.40 | .84 | +.18 | −.24 | +.86 | +.10 | .84 |
| Voc ..... | 5 | +.10 | −.26 | +.71 | +.13 | .60 | +.44 | −.62 | +.07 | +.14 | .60 |
| W ..... | 6 | +.53 | +.38 | +.20 | +.30 | .56 | +.63 | −.33 | −.07 | +.20 | .55 |
| F+ Per cent ... | 7 | +.48 | −.73 | −.36 | −.30 | .98 | +.14 | −.68 | +.70 | −.10 | .98 |
| Verb IQ ..... | 8 | −.24 | −.55 | +.50 | −.30 | .70 | +.08 | −.81 | +.06 | −.21 | .71 |
| Fv ..... | 9 | −.33 | +.86 | +.35 | −.08 | .98 | +.50 | +.22 | −.75 | −.35 | .98 |
| Sum Shading .. | 10 | +.37 | +.52 | +.29 | −.29 | .58 | +.60 | +.21 | +.05 | −.42 | .58 |
| Anxiety ..... | 11 | +.43 | +.55 | −.36 | −.34 | .73 | +.65 | +.24 | −.14 | −.50 | .75 |

TABLE 3

*Correlation Matrix, Group II*

| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Th | 1 | | −.223 | +.217 | −.467 | +.189 | +.389 | +.212 | +.156 | −.092 | +.201 | +.211 | −.003 | +.297 | +.193 | +.306 | +.350 | −.412 | +.109 | +.529 |
| Eco | 2 | −.223 | | −.346 | +.177 | −.298 | −.173 | −.573 | −.137 | −.378 | −.227 | −.294 | +.320 | −.128 | −.080 | +.128 | −.524 | +.494 | −.033 | +.060 |
| Aes | 3 | +.217 | −.346 | | −.526 | +.420 | +.311 | +.359 | +.053 | −.046 | +.407 | +.304 | −.257 | +.184 | −.015 | +.005 | +.519 | −.333 | −.186 | +.154 |
| Pol | 4 | −.467 | +.177 | −.526 | | −.348 | −.428 | −.197 | +.303 | +.012 | −.103 | −.035 | +.613 | −.332 | −.278 | −.209 | −.315 | +.468 | −.063 | −.289 |
| F1C | 5 | +.189 | −.298 | +.420 | −.348 | | +.288 | +.768 | +.339 | +.422 | +.830 | +.702 | −.241 | +.060 | +.348 | +.016 | +.460 | −.216 | +.222 | +.263 |
| F2S | 6 | +.389 | −.173 | +.311 | −.428 | +.288 | | +.180 | +.010 | −.048 | +.024 | +.150 | −.102 | +.477 | +.179 | +.227 | +.470 | −.128 | +.049 | +.621 |
| D | 7 | +.212 | −.573 | +.359 | −.197 | +.768 | +.180 | | +.344 | +.360 | +.721 | +.607 | −.311 | +.085 | +.330 | −.014 | +.486 | −.137 | +.272 | +.173 |
| Pd | 8 | +.156 | −.137 | +.053 | +.303 | +.339 | +.010 | +.344 | | −.046 | +.524 | +.599 | +.461 | −.159 | −.085 | −.348 | +.041 | +.054 | −.129 | −.037 |
| Pa | 9 | −.092 | −.398 | −.046 | +.012 | +.422 | −.048 | +.360 | −.046 | | +.222 | +.370 | +.031 | +.127 | +.266 | +.371 | +.285 | −.310 | +.052 | −.229 |
| Pt | 10 | +.201 | −.227 | +.407 | −.103 | +.830 | +.024 | +.721 | +.524 | +.222 | | +.885 | +.173 | −.138 | +.077 | +.051 | +.481 | −.030 | +.251 | +.269 |
| Sc | 11 | +.211 | −.294 | +.304 | −.004 | +.702 | +.150 | +.607 | +.599 | +.370 | +.885 | | +.329 | −.048 | −.008 | +.242 | +.438 | −.123 | +.119 | +.042 |
| Ma | 12 | −.003 | +.320 | −.257 | +.313 | −.241 | −.102 | −.311 | +.461 | +.031 | +.173 | +.329 | | −.193 | −.408 | +.022 | −.142 | +.265 | −.065 | .000 |
| Voc | 13 | +.297 | −.128 | +.184 | −.332 | +.030 | +.477 | +.085 | −.159 | +.127 | −.138 | −.048 | −.193 | | +.244 | +.489 | +.243 | −.220 | −.227 | +.202 |
| DSS | 14 | +.193 | −.080 | −.015 | −.278 | +.348 | +.179 | +.330 | −.085 | +.266 | +.077 | −.008 | −.408 | +.244 | | +.260 | −.080 | −.378 | +.048 | +.195 |
| VIQ | 15 | +.306 | +.128 | +.005 | −.209 | +.016 | +.227 | −.014 | −.348 | +.371 | +.051 | +.242 | +.022 | +.489 | +.260 | | +.182 | −.214 | −.094 | +.111 |
| EB | 16 | +.350 | −.524 | +.519 | −.315 | +.460 | +.470 | +.486 | +.041 | +.285 | +.481 | +.438 | −.142 | +.243 | −.080 | +.182 | | −.213 | +.090 | +.302 |
| Wv | 17 | −.412 | +.494 | −.333 | +.468 | −.216 | −.128 | −.137 | +.054 | −.310 | −.030 | −.123 | +.265 | −.220 | −.378 | −.214 | −.213 | | +.344 | +.097 |
| Wo | 18 | +.109 | −.033 | −.186 | −.063 | +.222 | +.049 | +.272 | −.129 | +.052 | +.251 | +.119 | −.065 | −.227 | +.048 | −.094 | +.090 | +.344 | | +.500 |
| FM | 19 | +.529 | +.060 | +.154 | −.289 | +.263 | +.621 | +.173 | −.037 | −.229 | +.269 | +.042 | .000 | +.202 | +.195 | +.111 | +.302 | +.097 | +.500 | |

TABLE 4

FACTOR MATRIX II (before rotation)

| | I | II | III | IV | V | $h^2$ |
|---|---|---|---|---|---|---|
| 1. Theoretical Interest | +.535 | +.322 | −.276 | −.188 | −.279 | .579 |
| 2. Economic Interest | −.535 | +.170 | −.311 | +.300 | −.167 | .530 |
| 3. Aesthetic Interest | +.520 | +.078 | −.162 | −.375 | +.312 | .541 |
| 4. Political Interest | −.572 | −.547 | +.111 | +.111 | −.343 | .769 |
| 5. F-1C (self-confidence) | +.808 | −.353 | −.053 | +.166 | +.088 | .816 |
| 6. F-2S (sociability) | +.515 | +.367 | −.307 | +.166 | −.148 | .544 |
| 7. MMPI Depression | +.754 | −.431 | +.198 | +.140 | +.227 | .865 |
| 8. MMPI Psychopathic Deviate | +.131 | −.536 | −.257 | −.283 | −.297 | .539 |
| 9. MMPI Paranoia | +.345 | −.277 | +.550 | +.115 | −.186 | .545 |
| 10. MMPI Psychasthenia | +.654 | −.602 | −.299 | −.052 | +.040 | .884 |
| 11. MMPI Schizophrenia | +.625 | −.605 | −.170 | −.172 | −.329 | .923 |
| 12. MMPI Hypomania | −.280 | −.367 | −.301 | −.083 | −.621 | .696 |
| 13. W-B Vocabulary | +.356 | +.488 | +.164 | +.080 | −.230 | .451 |
| 14. Digit Span Scatter | +.390 | +.270 | +.359 | +.316 | −.135 | .472 |
| 15. W-B Verbal I.Q. | +.285 | +.318 | +.181 | +.113 | −.423 | .407 |
| 16. Experience Balance | +.666 | +.106 | +.115 | +.113 | −.154 | .504 |
| 17. Vague whole responses | −.438 | −.297 | −.341 | +.483 | +.119 | .644 |
| 18. Ordinary whole responses | +.155 | −.202 | −.230 | +.484 | +.190 | .388 |
| 19. Animal Movement responses | +.428 | +.236 | −.568 | +.452 | −.078 | .772 |

TABLE 5

FACTOR MATRIX II (after rotation)

| | I | II | III | IV | V | $h^2$ |
|---|---|---|---|---|---|---|
| 1. Theoretical Interest | +.270 | +.638 | −.168 | +.161 | +.223 | .584 |
| 2. Economic Interest | −.369 | −.099 | −.229 | −.521 | +.256 | .535 |
| 3. Aesthetic Interest | +.371 | +.267 | −.302 | +.423 | −.260 | .547 |
| 4. Political Interest | −.098 | −.665 | +.290 | −.237 | +.436 | .782 |
| 5. F-1C (self-confidence) | +.802 | +.239 | +.222 | +.011 | −.260 | .817 |
| 6. F-2S (sociability) | +.239 | +.662 | −.124 | −.148 | .000 | .533 |
| 7. MMPI Depression | +.724 | +.087 | +.375 | +.133 | −.427 | .872 |
| 8. MMPI Psychopathic Deviate | +.515 | −.252 | −.035 | +.134 | +.440 | .542 |
| 9. MMPI Paranoia | +.219 | +.021 | +.693 | +.163 | −.067 | .560 |
| 10. MMPI Psychasthenia | +.936 | −.033 | −.003 | +.062 | −.029 | .882 |
| 11. MMPI Schizophrenia | +.869 | +.013 | +.195 | +.174 | +.310 | .920 |
| 12. MMPI Hypomania | +.140 | −.270 | .000 | −.182 | +.767 | .714 |
| 13. W-B Vocabulary | −.114 | +.630 | +.189 | +.064 | +.007 | .450 |
| 14. Digit Span Scatter | −.023 | +.467 | +.472 | −.047 | −.203 | .485 |
| 15. W-B Verbal I.Q. | −.062 | +.512 | +.318 | .000 | +.202 | .408 |
| 16. Experience Balance | +.358 | +.527 | +.286 | +.082 | −.089 | .502 |
| 17. Vague whole responses | −.001 | −.430 | −.160 | −.658 | −.019 | .644 |
| 18. Ordinary whole responses | +.316 | −.023 | −.011 | −.472 | −.258 | .390 |
| 19. Animal Movement responses | +.353 | +.558 | −.258 | −.528 | −.022 | .782 |

TABLE 6

*Significant Factor Loadings*

| Test | Variable | Loading | Matrix |
|---|---|---|---|
| | *Factor A, Delay and Inhibition* | | |
| MMPI | Psychasthenia | 0.94 | II |
| MMPI | Schizophrenia | 0.87 | II |
| Rorschach | Special F Plus | 0.86 | I |
| Flanagan | F-1C (Lack of) Self-confidence | 0.80 | II |
| Rorschach | Human Movement | 0.75 | I |
| MMPI | Depression | 0.72 | II |
| Rorschach | F Plus Per Cent | 0.70 | I |
| MMPI | Psychopathic Deviate | 0.52 | II |
| Allport | Aesthetic Interest | 0.37 | II |
| Rorschach | Animal Movement | 0.35 | II |
| Rorschach | Experience Balance | 0.35 | II |
| Rorschach | Vague Form Responses | −0.75 | I |
| Allport | Economic Interest | −0.37 | II |
| | *Factor B, Verbal Adjustment* | | |
| Wechsler | Verbal I.Q. | 0.81 | I |
| Rorschach | F Plus Per Cent | 0.68 | I |
| Flanagan | F-28 (Independence vs.) Social | 0.66 | II |
| Allport | Theoretical Interest | 0.64 | II |
| Wechsler | Vocabulary | 0.63 | II |
| Wechsler | Vocabulary | 0.62 | I |
| Rorschach | Experience Balance | 0.53 | II |
| Wechsler | Verbal Scale (I-C-S) | 0.51 | II |
| Rorschach | Animal Movement | 0.56 | II |
| Wechsler | Digit Span Scatter | 0.47 | II |
| Rorschach | Human Movement | 0.45 | I |
| Allport | Political Interest | −0.66 | II |
| Rorschach | Color (Sum C) | −0.50 | I |
| Rorschach | Vague Wholes | −0.45 | II |
| | *Factor C, Emotional Drive (Surgency)* | | |
| Rorschach | Number of responses (r) | 0.71 | I |
| Rorschach | Color (Sum C) | 0.69 | I |
| Rorschach | Vague Wholes (Wv) | 0.66 | II |
| Rorschach | Total W | 0.63 | I |
| Ratings | Anxiety | 0.65 | I |
| Rorschach | Shading | 0.60 | I |
| Rorschach | Animal Movement | 0.53 | II |
| Allport | Economic Interest | 0.52 | II |
| Rorschach | Vague Form (fv) | 0.50 | I |
| | *Factor C, Emotional Drive (Surgency)* | | |
| Rorschach | Ordinary Wholes (Wo) | 0.47 | II |
| Wechsler | Vocabulary | 0.44 | I |
| Allport | Aesthetic | −0.42 | II |
| | *Factor D, Anxiety* | | |
| MMPI | Paranoia | 0.60 | II |
| Ratings | Anxiety | 0.50 | I |

TABLE 6—Continued

*Significant Factor Loadings*

| Test | Variable | Loading | Matrix |
|---|---|---|---|
| Wechsler | Digit Span Scatter | 0.47 | II |
| Rorschach | Shading | 0.42 | I |
| MMPI | Depression | 0.38 | II |
| Rorschach | Vague Form (Fv) | 0.35 | I |
| | *Factor E, (Hypo) Manic-Depression* | | |
| MMPI | Hypomania | 0.77 | II |
| MMPI | Psychopathic Deviate | 0.44 | II |
| Allport | Political Interest | 0.44 | II |
| MMPI | Depression | −.043 | II |

The five factors of the second matrix were rotated 16 times, although, again, simple structure became evident somewhat earlier; the results are shown in tables 4 and 5. One of the five factors obtained from this battery is limited to the questionnaires, not appearing in the Rorschach data.

The following identifications of factors from the two matrices were made upon the basis of similar loadings on common items (the principle of proportional profiles), supported by consideration of the psychological nature of items peculiar to one matrix or the other:

*Factor A.* Factor III of the first matrix and factor I of the second matrix are paired on the basis of their common loading of the Rorschach movement responses with zero loading on the intelligence tests; this identification is strongly supported by the introversion-extroversion context of every item weighted by this factor.

*Factor B.* The equivalence of the second factors in the two matrices is indicated here by the very similar loadings on the intelligence test items.

*Factor C.* The common *W* variables identify factor I of the first matrix with factor IV of the second.

*Factor D.* The common presence of clinical anxiety indicators, including the clinical rating of anxiety, identify factor IV of the first matrix with factor III of the second.

*Factor E.* This is the questionnaire factor unique to the second matrix.

Inspection of the communalities reveals clearly that the present factors account for almost all of the significant variance

in the majority of the measures used, the communalities approaching the reliability coefficients in many cases. Whole responses are conspicuous by their deviation from this pattern with much of their variance being left unexplained.

The two communalities of 0.98 in the first matrix are not to be taken too seriously as tetrachoric correlations tend to overestimate the Pearsonian coefficient; nevertheless, the communalities amply justify the treatment of the Rorschach test as a psychometric instrument.

The loading above 0.35 are shown for each factor in table 6. The names given the factors are explained in the following chapter.

*The Psychological Nature of the Factors.* From inspection of the variables with significant loading on factor A, one would be tempted to deny the possibility of any psychological meaning inhering in the factor. Psychasthenia, schizophrenia, "intellectual control," lack of self-confidence, and depression are not synonymous terms in the lexicon of the psychologist. Yet, this difficulty is more apparent than real; the factorial findings are in accord with a theoretical approach to personality questionnaires and neurotic inventories which has found considerable currency among psychologists within the last few years.

Most personality inventories have been constructed upon the assumption that the answers to questionnaire items may be taken at face value as indicators of the presence or absence of the symptom implied by the item. Thus, such a question as "Are you afraid of high places?" is, as we have seen, often assumed to indicate the presence or absence of a phobia. We have all been fearful in high places at one time or another; when we answer "no" to the item, we are implicitly making a normative judgment: "Compared to the average person, I am not especially disturbed by high places," If, on the other hand, we are *scrupulously* honest and adhere to an "absolute" set of norms, the question must be answered "Yes." *It is apparent that responses to questionnaire items are a function of the normative, evaluative, and perceptual systems of the subject as well as a function of the content of the item.*

It is rather amazing that psychologists have not devoted more attention to this phenomenon. Correlations of 0.95 have been re-

ported between scales purporting to measure "introversion" and "neuroticism"; yet, instead of investigating the validity of the labels pinned on the tests, psychologists have sometimes blithely identified normality with "extraversion." The developers of the Minnesota Multiphasic Personality Inventory have at least been cautious in regard to the high correlations between their variables and have explained them away as best they could. Thus, the marked correlation between "depression" and every other one of their scales except "hypomania" has been rationalized by the assumption that some depression is present in every neurotic syndrome (Hathaway and McKinley, 1943). Their 0.85 correlation between "psychasthenia" and "schizophrenia" is discounted by explaining that the schizophrenic scale appears to have some value when extremely high and by the explanation that the scale is only tentatively included in the test.

The MMPI workers ultimately recognized the existence of an unexplained "plus-getting" factor, but, instead of investigating its psychological nature, simply attempted to work out correction factors which would have the effect of partialling the factor out of the scale. These attempts have been unsuccessful.

Now let us turn to the Rorschach data for a moment. Table 6 shows *F plus per cent*—the predominance of accurately perceived forms—to be a function of both factor A and factor B, the latter associated with intelligence, while *special F plus*—finely articulated, definitive, and well elaborated percepts—is almost entirely a function of factor A.

Again, in the Flanagan measure, we see this factor defined as the lack of self-confidence. Now let us present the rationale which appears to acount for all of these definitions of the factor.

Factor A may be termed the inhibition of spontaneity. Rapaport has developed this concept in his discussion of movement responses under the name of "delay":

> The fact that certain people are more inclined to "think things out" and others more to follow their impulses and "act out", seems to have served as the basis for the introvert-extrovert dichotomy. However, such dichotomous thinking neglects the fact that delay of impulse, whether instinctual or affective, is the fundamental condition for human thinking; it is the prerequisite of every thought, and indeed gave birth to thought. In other words, the development of the Ego and its thought processes represent a progressive mastery over impulses. A delay between impulse and its discharge must come

about so that during this delay the reality situation may be "tested", and the least dangerous way of reaching the goal be discovered. Thus, thought serves the impulse but is rendered possible only by a delay of its discharge. When the Ego is more fully developed and the individual is able to think beyond the immediate tension-experience, an impulse may not only be delayed but curbed and deflected from its goal, or even totally rejected, for the benefit of the organism as a whole.

From a description of the persons in whom M responses are found to prevail, it would appear that they excel in this ability for delay—and might even show pathological extremes of delay in the form of action-paralyzing obsessive doubts or phantasies. Thus, the process which is presumed to lead to the M response parallels the process which actually leads to that type of behavior in every-day life to which the response apparently refers. We have also pointed out that the M response implies an anticipation of the most stable spatial relationships of the areas chosen; and this characteristic also is shared by thought processes in general, whose function is to anticipate various pathways toward the object of the impulse and the dangers implied in pursuing them.

Therefore, in normals the M response should be considered to indicate the level of natural endowment—insofar as natural endowment comes to expression in a wealth and vividness of ideation—a readiness to make anticipations, and a versatility and flexibility of perceptual and associative processes in general. That a too great prevalence of M responses indicates a decline of spontaneity, an abating of the intensity of drives and their striving for expression, is patent: with too much delay the impulse is impoverished. It is also patent that the M response does not refer to a category of psychic make-up, or to a reaction-tendency present in some individuals but absent in others: movement responses refer rather to a propensity present in all individuals, and varying only in its strength and the context in which it is set. With these views accepted, the term *introversion* can be forgotten. The M response is an indicator of natural endowment and of the intensity of ideational activity: in different "normal" contexts, these may show up as creativeness or as an inclination to be ruminative; in different neurotic contexts, they may show up in the form of obsessions and/or phobias; in the context of psychoses, they may show up as delusion-formation.

This concept has far-reaching implications. Accepting this theoretical formulation enables one to account for the high intercorrelations between various questionnaires and renders suspect the labels applied to many current questionnaires.

In this study, the "delay factor" will be used as a name for factor A. This avoids the ambiguity inhering in the use of the term "inhibition," which is used to refer to the inhibition of thought processes as well as inhibition of responsivity. Factor A decidedly does not imply thinking inhibition; rather, it implies the precise opposite. "Intellectual control," another possibility

based upon Klopfer's use of the term in Rorschach analysis, invites too much confusion with factor B to be of value here.

Factor B appears to be verbal intelligence and its personality correlates. The presence of Flanagan's "Independence versus Sociability" and Allport's "Theoretical Interest" here may disturb some, since both are concepts not usually considered to be closely related to intelligence. This point will be considered in detail elsewhere. It is enough to indicate here that this relationship is of crucial significance for the science of personality and does not appear to be an artifact.

It is necessary to justify the contention above that factor B represents verbal intelligence (Thurstone's "V") rather than "g" or general intelligence. One indication of this is the factor pattern of "digit-span scatter"—the relationship between the essentially verbal tests and Wechsler's digit-span subtest. As predicted by clinical findings, the lowering of the digit-span weighted score relative to the verbal level indicates the presence of anxiety (see the discussion of factor D below); however, this relationship also has a significant loading on factor B. Thus, we see that factor B indicates a differential between verbal intelligence and digit-span, also an intelligence measure. In other words, the difference between digit-span and the verbal measures may be due either to an impairment of digit-span or a verbal superiority. In addition to this, we have Thurstone's (1941) evidence that vocabulary tests similar to the Wechsler measure are the best available measures of "V."

Factor C is of special interest since it appears to be identical with Thurstone's "W" and Cattell's "surgency." Cattell (1946) has suggested this identification on the basis of the Rorschach variables Thurstone included in his study of perception previously cited. Additional evidence for this identification of a personality variable with a "primary mental ability" appears in a recent study by Gewirtz (1948) in which word fluency correlates with precisely the variables one would predict from the weighting this factor has on the Rorschach color scores in the present study. Cattell has advocated the use of "W" as a convenient measure of "surgency" or, as the present writer prefers to designate the factor, emotional drive.

Factor D, with loadings on MMPI Paranoia, Anxiety ratings,

digit-span scatter (a clinical measure of anxiety), shading (the Rorschach reflection of anxiety), MMPI Depression, and vague form (a second Rorschach indicator of anxiety), appears clearly to be a specific anxiety factor.

Factor E, appearing only in the questionnaires, is plausibly designated the manic-depressive factor in view of its positive loadings on MMPI Hypomania and Psychopathic Deviate, Allport's Political Interest, and its negative loading on the MMPI Depression scale.

*The Psychological Implications of the Study.* We have seen that the concept of a delay in reactivity may account for the constant and plaguing correlations between personality questionnaires purporting to deal with a multiplicity of variables. Obviously, many measures of this factor are available. The MMPI variable, psychasthenia, with the astounding saturation of 0.94, is the purest of our measures of factor A. Unfortunately, this is a questionnaire and, as such, subject to faking and distortion. Another "pure" measure of factor A is the "special F plus" Rorschach score; here, the problem of faking is absent, but the troublesome necessity for subjective appraisal by the examiner is introduced.

At the present time, no satisfactory, "foolproof" method of measuring the trait suggests itself. A selection of items from the multiphasic "Psychasthenia" and "Schizophrenia" scales and Flanagan's F-IC might be made which would avoid items with a "neurotic" taint and, hence, be less likely to be distorted by the subject. If necessary, the Rorschach test could be used as a further check. For the present, the use of the psychasthenic items from the Multiphasic would seem to be a fairly adequate stop-gap measure.

The measurement of factor B does not appear to offer any difficulty; rather, it suggests a goal toward which clinical psychology should strive: the measurement of personality in the guise of "abilities"—a perfect "projective technique" since, within our culture, high scores on "intelligence tests" are universally approved. The vocabulary tests of Thurstone, Wechsler, and Terman all should prove adequate measures of factor B, given a satisfactory method of making "ipsative" comparisons. The relationship between "V" and the individual's average score

on a fairly wide range of non-verbal measures of intelligence such as the Wechsler performance scale or Thurstone's PMA scales should provide a good index to the ideational (or over-ideational) pattern of adjustment.

As we have seen, word fluency tests are already being utilized to measure factor C, with the same advantages as the measurement of factor B as an ability. The need for "ipsative" comparisons can be handled as indicated above.

Factor D represents a much more difficult problem in measurement. Apparently, our clinical estimates of anxiety confuse the anxiety factor with emotional drive or surgency; this is also true of the Rorschach shading responses as a measure. The digit-span scatter apparently depends almost equally upon factors B and D for its variance, and an equation, such as D = DDS — V, in which the influence of "V" is partialed out, should give us an objectively determined univocal estimate of factor D. The two-factor nature of anxiety, as clinically determined, deserves further investigation.

Factor E is not satisfactorily reflected in our variables. The MMPI Hypomania scale gives a fairly adequate questionnaire-type measure of the variable, but MMPI Depression is a very poor measure of the negative pole of this factor.

Will it eventually be possible to measure most of the significant personality traits in the guise of abilities? Although it is impossible to answer this question with confidence, certain considerations are encouraging. Most of the variables we are interested in represent ways in which the organism adapts to the pressures of the environment; these adjustive devices represent, in a very real sense, problem-solving behavior and, as such, should lend themselves to objective measurement.

Will it be possible to abandon the Rorschach test and similar cumbersome and time-consuming projective techniques? Eventually, perhaps, but certainly not yet. The psychometric approach utilized in the present study does not pretend to encompass the entire range of information about a subject available in a detailed study of the Rorschach record; yet, it would seem reasonable to expect that objective tests could be standardized which would give as much information about a person as, say, the Rorschach test in the hands of a novice, and would have the decided ad-

vantage of being amenable to group administration and scoring. This would be a decided boon to the overworked clinician.

In many respects, the most important aspect of the present study is the attempt to demonstrate the integrative power of factorial studies which cross the artificial boundaries in the human personality set up as a result of the divergent interests and theoretical viewpoints in psychology. The five factors presented here account for a major portion of the variance of 26 variables, each with its own label. The present results support Jastak's statement:

> The broadening schism between factorial and clinical psychology is another hurdle to be cleared. Statistical experts invent highly complex mathematical techniques whose clinical applications are distinctly limited. Clinicians, on the other hand, engage in progressively refined studies of personality dynamics without adequate statistical proof for the far-reaching conclusions they make. This impasse may, of course, be resolved by filling in the gaps of either system. The hunches and intuitions of the clinician must be supplemented by precise mathematical treatment to make them objective and generally applicable. The elaborate computations of the statistician must be supplemented by clinical insight to endow them with the necessary life values. More teamwork between factorist and clinician will benefit all psychologists. . . . The various techniques of factorial and clinical psychology are equivalent insofar as each of them yields fractionally correct results in personality analysis. A rational combination of most current methods of measurement into one comprehensive system will produce a more complete and a more precise science of the personality than any single technique by itself.

It is difficult to see how psychology can afford to limit itself to investigation of "intelligence," "ability," "temperanment," and the like as if the human psyche were rigidly compartmentalized. The beginnings of a science of personality are in sight and, as frequently occurs in science, the greatest advances may well be made in the borderline areas between disciplines and subdisciplines.

The following conclusions may be drawn from the present study:

1. With appropriate modifications, the Rorschach test may be utilized as a psychometric instrument.

2. Four factors satisfactorily account for the variance of the Rorschach scores used in this study, with the exception of *W*, which retains a considerable amount of specific variance.

3. The concept of "delay" offers a promising theoretical explanation of the disturbingly high intercorrelations among questionnaires designed to measure presumably independent traits.

4. The present study lends support to the hypothesis that significant relationships exist among traits variously denominated "interests," "abilities," and "personality traits."

5. Measures of factorial "abilities" may prove useful in the measurement of personality traits, apparently promising increased objectivity, simplicity of measurement, and relative freedom from distortion.

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# PRODUCTION OF HEPATIC ABNORMALITIES IN CHICK EMBRYOS BY THE YOLK SAC CULTURE OF MOUSE TUMOR*

IRVING GALINSKY†

The problem of hepatic changes caused by non-hepatic cancers is important because the liver is very closely linked with the control of many metabolic functions. One of the most frequently reported changes is an increase in the per cent water content in the liver. In humans, Abels and associates, and Ariel and Shahon have found liver dysfunction resulting from cancers of the biliary system and also from gastrointestinal cancers. They conclude that some mechanism other than anatomical obstruction contributes to hepatic dysfuction and that the cancer is the factor that disrupts hepatic function.

It has been reported by Taylor, Armstrong, Ham *et al.*, that when certain mouse mammary carcinomas are grown in the yolk sac of eggs, they adversely effect the liver of the chick embryo. However, in control eggs with no tumors, a small per cent of chick embryos are found with abnormal livers. With this in mind it was decided to undertake some preliminary experiments to determine statistically, whether the tumors caused a significant increase in the numbers of abnormal livers.

## *Materials and Methods*

The tumor used in these experiments is a mammary carcinoma from a dba mouse and was obtained from Dr. A. Taylor at The University of Texas where it had been grown continuously in eggs for 146 generations. The tumor was reinoculated into a dba mouse and taken to Baylor University College of Medicine where it was grown continuously in eggs for 23 generations before being utilized for these hepatic studies. Dr. Taylor lists this tumor by the letters RC.

The technique of growing the tumor in the egg yolk sac is essentially that developed by Taylor. A portion, 0.6 cc., of the macerated tumor is suspended in 4 ccs. saline and 0.4 cc. of the suspension is injected through the air sac

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into the yolk sac of an embryonated chick egg which had been incubated for 4 days.

In order to determine whether only viable tumor tissue had the ability to produce liver damage, both tumor and liver tissues which had been destroyed by freezing and thawing were also injected into the egg yolk sacs. The method of killing the cells was to freeze them at — 10° C. and then thaw them at 28° C. These tissues were then macerated, suspended in saline, and injected into the yolk sac in exactly the same manner and concentration as that used for the viable tumor tissue. The tissue was kept frozen until ready for use.

The living and frozen materials were sectioned and examined for signs of destruction. To further determine viability, suspensions of frozen tumor were injected subcutaneously into mice and also into eggs. The mice were examined for a period of 6 months and the eggs for 16 days.

The following tissues were compared as to their ability to produce liver damage: viable tumor, frozen tumor, green chick livers from tumor bearing eggs, normal chick livers, and green livers from non-tumor bearing eggs.

*Observations.* Abnormal livers can be divided, upon gross examination, into 3 general classes: (1) normal colored livers with yellow portions of varying size, (2) green livers, and (3) green livers with yellow portions of varying size.

The first sign of an abnormality is usually a small yellow spot or thin yellow band on the liver margin. However, livers may turn green without showing any signs of having had yellow portions. For the purpose of statistical comparison, green livers and green livers with yellow portions are grouped together and referred to as green livers.

Histological sections of the liver disclose various degrees of derangement corresponding to the gross picture. The yellow areas consist of sharply defined regions of necrosis of liver cells. They are usually situated at the perhiphery of the liver and consist of a zone of cells which stain lighter red than the normal tissue immediately adjoining it. The cellular outlines are preserved, but the individual cells, are separated from one another in contrast to the normal cords of cells in the adjacent tissue. Nuclear detail is usually lost in this zone. Farther in the necrotic focus the cells take on a still brighter red color and are still separated slightly from one another with only a hint of the cord-like arrangement characteristic of normal liver. In this zone the nuclei are quite distinct and appear as central, round, deep blue staining structures. Most of the sinusoids in this area are empty but occasionally contain a few cells and nuclear debris. In some

portions of the lesions there is quite complete disintegration of the liver cells which then appear as granular masses of reddish-blue staining material without cellular or nuclear detail. Along with this granular debris and perhaps associated with it are darker masses of granular material, frequently aggregated in irregular masses so deeply stained that they appear almost black.

In the green portions of the liver, there are present minute masses of homogeneous red-brown staining material between some of the liver cells. This finding is fairly prominent in some portions of the liver and may represent bile stasis.

The extent of liver damage is not entirely dependent upon the size of the tumor growing in the yolk sac. Extreme liver damage may be found in eggs with only 0.1 gram tumor while some with 2 gram tumors may have normal livers and vice versa.

Table 1 contains the data on 58 eggs in which the same tumor

TABLE 1

*Effect of Yolk Sac Grown Mouse Mammary Carcinoma on Chick Embryo Livers at Various Incubation Ages*

| Embryo Age (days) | Per cent Normal Livers | Per cent Abnormal Livers | | |
|---|---|---|---|---|
| | | Red+Yellow | Green±Yellow | Total |
| 14 | 31.25 | 50.00 | + 18.75 | = 68.75 |
| 16 | 36.00 | 8.00 | + 56.00 | = 64.00 |
| 18 | 35.30 | 11.80 | + 52.90 | = 64.70 |

was grown. From this it can be seen that while there is the same proportion of normal to abnormal livers in 14, 16, and 18 day embryos, there is a decided shift from the red and yellow livers at 14 days to the green liver group at 16 days. From here until at least 18 days the proportion remains the same. Observations for the remaining experiments were made at 16 days since the embryos had not yet begun to die in large numbers and the maximum numbers of easily seen green livers were present.

A total of 920 living embryos were examined in 18 different experiments. When saline injected eggs were compared with non-injected eggs it was found that there was no increase in the number of abnormal livers present (Table 2). Saline injected eggs were then used as controls for comparison with the other injected materials.

Frozen normal livers and the green livers which are sometimes found in non-tumor bearing eggs were ineffective in

causing liver damage (Tables 3 and 4). There was a very significant increase in the number of abnormal livers found in eggs in which live tumor was grown in the yolk sac. This was also true when frozen tumor and green livers obtained from

TABLE 2

*Comparison of Non-Injected and Saline Injected Non-Tumor Eggs*

| Injected Material | No. Eggs | No. Normal Livers | No. Abnormal Livers | $X^2$ |
|---|---|---|---|---|
| Non-injected | 19 | 17 | 2 | .... |
| Saline | 17 | 16 | 1 | 0.734* |

P(.50)–0.455 ; P(.30)–1.074.
* No significant difference.

tumor bearing eggs were injected into the yolk sac. These latter two materials cause about the same per cent increase in abnormal livers. When tumors which produced a very high per cent of abnormal livers were frozen, thawed and injected into the yolk sac, there was a great increase in the numbers of abnormal livers but this was never as high as that found with the original live tumor. These data are summarized in table 3 where they are compared statistically using the Chi-Square test.

The effectiveness of the slow freezing and thawing method for destroying cells could be seen in histological sections where

TABLE 3

*Comparison of the Ability of Various Tissues in Producing Abnormal Livers*

| Injected Material | No. Eggs | No. Normal Livers | No. Abnormal Livers | $X^2$ | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Saline | 263 | 226 | 37 | .. | .. | .. | .. | | |
| Frozen Normal Livers | 122 | 104 | 18 | 0.048 | | | | | |
| Frozen Green Livers from Tumor Eggs | 76 | 38 | 38 | | 81.189* | | | .. | .. |
| Frozen Tumor | 92 | 43 | 49 | | | 116.936* | | 0.391 | |
| Live Tumor | 170 | 55 | 115 | | | | 403.592* | 21.176* | 14.138* |

P(.001)–10.827 ; P(.01)–6.635 ; P(.05)–3.841 ; P(.10)–2.706 ; P(.50)–0.455 ; P(.80)–0.064.
*Significant difference.

the cells were observed to be ruptured. When frozen and thawed material was injected into the yolk sac there was never any sign of growth present when the eggs were opened. Similarly, a subcutaneous injection of this material into mice resulted in no growth even after 6 months. Live tumor cells produced growths in a short time.

*Discussion.* In the yolk sac technique of growing tumors there is no physical contact between the tumor and the chick embryo. The only connection is via the blood stream. In order to influence the liver of the chick embryo, the tumor must do so by some substance or substances given off into the bloodstream. The possibility that these are the products of necrosis is ruled out by the healthy appearance of the tumor nodules both on gross and histological examination.

TABLE 4

*Comparison of Saline and Frozen Green Livers from Normal Eggs Injected into Non-Tumor Eggs*

| Injected Material | No. Eggs | No. Normal Livers | No. Abnormal Livers | $X^2$ |
|---|---|---|---|---|
| Saline | 27 | 21 | 6 | .... |
| Frozen Green Livers from Normal Eggs | 20 | 13 | 7 | 1.899* |

P(.10)–2.706; P(.50)–0.455.
* No significant difference.

Frozen normal livers, and green livers which are sometimes found in eggs without tumors do not cause liver abnormalities, while viable tumor, frozen tumor, and green chick livers from eggs with tumors do. There thus seems to be something present in the latter group which is not present in the former. This substance is not destroyed by the freezing and thawing within a range of —10° C. and 28° C. The tumor cells evidently release this substance and it accumulates in the liver of the chick embryo (whether it also accumulates in other tissues is not known at present). When these livers are macerated and injected into the egg yolk sacs, the substance present can again produce abnormal livers. The fact that green livers are occasionally found in eggs without tumors suggests that the liver damage caused by the tumor is not a highly specific one.

Greenstein and co-workers have shown that there is a great decrease in liver catalase when cancer tissue is introduced into the animal. They also point out that this decrease in liver catalase is not produced by rapidly growing cells (regenerating liver) or embryonic cells (pregnancy or implanted embryonic growths). Greenstein suggests a tumor activity directed against the synthesis of hematoporphyrin prosthetic groups.

Taylor suggests that the toxic effect of a yolk sac tumor is mediated through a tumor agent which is given off into the blood stream and is circulated through the chick embryo. Armstrong and Ham are of the opinion that anemia is the basic pathological lesion produced and that all other lesions seen in the chick are consequent to it.

The present work seems to favor the view that some substance from the tumor is circulated through the chick and thus causes the damage. Some tumor cells seem to be more active in the production of this substance than others as evidenced by the fact that small tumors are sometimes more destructive than large tumors. These tumors can be selected, over many generations, for an increased potency in causing damage and eventually a strain of cells can be obtained which will cause the death of the embryo at a very early age. The tumor does not appear to undergo any morphological or histological changes.

The significant decrease in the liver damaging ability between the viable tumor and its frozen counterpart suggests that live cells are active in the production of this substance. Dead tumor cells contain a certain amount of this substance but are not capable of producing any more, which would account for the decreased effectiveness of the dead material.

## *Summary*

A statistical comparison was made as to the liver damaging ability of various tissues injected into the yolk sac of chicken eggs. The production of abnormal livers by yolk sac-grown mouse tumor was confirmed in these studies. It was also found that homogenates of inviable tumor, and green chick livers found in eggs with tumors, were also capable of producing abnormal livers. Saline, normal livers, and green livers occasionally found in eggs without tumors, were not capable of producing abnormal

livers. The liver damaging agent appears to be some substance produced by the tumor.

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# STUDIES ON THE *IN VITRO* ACTION OF COMPOUND 48–80, A FORMALDEHYDE CONDENSATION PRODUCT, ON RAT MAST-CELLS*

L. C. U. JUNQUEIRA AND B. BEIGUELMAN

A marked action of Compound 48–80 promoting a vigorous extrusion of rat mast-cell granules was described by Motta, Beraldo, and Junqueira (1953). Compound 48–80 is an extremely active histamine liberator (Paton, 1951) and seems to us, a useful tool to study certain aspects of mast-cell physiology. The designation, Compound 48–80, was given by the chemists who first prepared it, to a complex condensation product of P-methoxy-phenethyl-methylamine with formaldehype.

As the functions of these cells are the subject of a series of controversial papers, data on the metabolism and physiology of mast-cells would be of interest. In this paper we present results obtained studying the influence of concentration, temperature, pH and metabolic inhibitors on the action of Compound 48–80 on mast cells. Furthermore, data obtained with metabolic inhibitors might contribute to characterize the different responses of the mast-cells; the so-called "response repertory" of Weiss (1949).

## *Material and Methods*

Adult Wistar rats, killed with a blow on the head were used throughout. All experiments were performed *in vitro*, submitting fragments of rat mesentery to the different conditions studied. Fragments of approximately 1 cm.$^2$ were carefully cut out and immersed in saline solution (0.9 per cent) plus 10 per cent phosphate buffer (0.15 M, pH 7.4), containing the substances or in the conditions studied.

To observe the action of temperature, fragments were immersed in the above mentioned solution at different temperatures during 10 minutes; Compound 48–80 (1/20,000 final concentration) was added and 10 minutes after the fragments were fixed and stained in a 10 per cent formaline solution containing 1 per cent toluidine blue and 0.1 per cent acetic acid.

To study the action of pH, Michaelis veronal-acetate buffers (0.1 M) were used. Incubation of mesentery fragments was performed at 37° C. during

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10 minutes. Compound 48–80 was added and 10 minutes after, fixed and stained as described.

The action of metabolic inhibitors was essayed in different concentrations. Conditions of the experiment are detailed in table 2. The influence of the lack of oxygen was studied incubating fragments in Warburg flasks, in nitrogen atmosphere and in the presence of red phosphorus during 10 minutes, after which 48–80 (1/20,000 final concentration) was added.

The effect of the addition of glutathione and adenosinetriphosphate (ATP) was studied in some cases after the incubation in some inhibitors, in order to verify the probable reversibility of the inhibitory action. Glutathione was used at the concentration of $1 \times 10^{-2}$ and ATP at 0.02 N.

The action of Compound 48–80 on the mast-cells morphology was also studied by aid of phase microscopy in fresh mesentery fragments. Successive photomicrographs were made before, during and after the action of 48–80, in order to study the evolution of the cellular modifications promoted by this substance.

A minimum of five experiments was performed in all cases.

## *Results*

Experiments performed with different concentrations of Compound 48–80 showed that it is extremely active, promoting a marked granule extrusion *in vitro*, in concentrations up to 1/500,000. The results obtained at different temperatures (10, 15, 20, 25, 30 and 37° C.) showed that below 25° C. inhibition of the extrusion activity of Compound 48–80 occurs. The influence of different pH's is presented in table 1 and it is evident that Compound 48–80 acts only in neutral and alkaline medium. Anaerobiosis does not prevent the action of Compound 48–80

TABLE 1

*Influence of pH on the Action of Compound 48–80 on Rat Mast-Cells "In Vitro"*

| pH | Buffer plus 48–80 (1/20,000) | Control (only buffer) |
|---|---|---|
| 2.88 | No extrusion | No extrusion |
| 3.52 | " " | " " |
| 4.56 | " " | " " |
| 5.36 | " " | " " |
| 6.26 | " " | " " |
| 7.18 | Extrusion | " " |
| 7.94 | " | " " |
| 8.40 | " | " " |
| 9.27 | " | " " |

while the lack of oxygen alone, in the controls, does not induce morphological modifications.

One may see observing table 2 that p-chloromercuribenzoate (p-Cl Hg benzoate), o-isodosobenzoate, iodoacetate, 2,4 dinitrophenol, arsenite, cyanide, urethane and uranil nitrate in the concentrations used, inhibited the extrusion of the mast-cells granules. Malonate and fluoride had no action. Azide as a cytochrome oxidase inhibitor, was not used because it acts efficiently

TABLE 2

*Influence of Metabolic Inhibitors on the Action of Compound 48–80 on Rat Mast-Cells "In Vitro"*

Mesentery fragments were incubated in the inhibitor dissolved in saline-buffer solution during 15 minutes at 37° C. and Compound 48–80 added (1/20.000 final concentration), for another 10 minutes. Fixation and staining as described in text.

| Inhibitor | Concentrations used Maximal | Minimal | Granules extrusion |
|---|---|---|---|
| p-Cl Hg benzoate | 0.33 x $10^{-2}$ M | 1 x $10^{-3}$ M | Absent |
| o-Iodosobenzoate | " " | " " | " |
| Iodoacetate | " " | 0.16 x $10^{-2}$ M | " |
| 2, 4 Dinitrophenol | " " | 0.12 x $10^{-3}$ M | " |
| Arsenite | " " | 1 x $10^{-3}$ M | " |
| Cyanide | " " | " " | " |
| Malonate | " " | ..... | Present |
| Fluoride | " " | ..... | " |
| Urethane | 0.06 M | ..... | Absent |
| Uranil nitrate | 1 x $10^{-3}$ M | 0.5 x $10^{-3}$ M | " |

at an acid pH (Stannard and Horecker, 1948), condition in which Compound 48–80 is inactive on mast-cells. Cyanide was used as a cytochrome oxidase inhibitor (Lockart and Potter, 1941; Potter, 1941; Horecker and Kornberg, 1946).

It is interesting to observe that the first three inhibitors in table 2, are considered to act on —SH groups (Hellermann, 1937; Hellermann, Chinard, and Ramsdell, 1941; Barron and Singer, 1943, 1945; Singer and Barron, 1945) while arsenite dinitrophenol and urethane probably act on phosphorilative mechanism of the cell metabolism (Hotchkiss, 1944; Clifton, 1946; Loomis and Lipmann, 1948; Florijn and Smits, 1949).

In order to further study this aspect, glutathione and ATP were added before and after the action of some inhibitors in an attempt to verify the reversibility of the inhibition promoted

by the above mentioned —SH group blocking substances, and by the phosphorilation inhibitors.

In the glutathione experiments, the mesentery fragments were immersed in either p-Cl Hg benzoate, iodoacetate or o-iodosobenezoate for 10 minutes at 37° C. They were then washed in the saline-buffer solution in order to remove the excess of inhibitor, and immersed in glutathione for 10 minutes and then 48–80 was added, acting during more 5 minutes. We could observe that granule extrusion occurred, *i.e.*, the glutathione reactivated the mast-cells permitting 48–80 to exert its action. Controls washed in saline only, did not react to the action of 48–80. If fragments are incubated first in glutathione for a greater providement of —SH groups, and after in the inhibitors these have no effect, since Compound 48–80, when added extruded the mast-cell granules.

Due to the fact that 2,4 dinitrophenol, arsenite and urethane have been described as acting on phosphorilative processes. ATP was added after immersion in several inhibitors in order to observe if its presence would reactivate the mast-cells. It was observed that ATP did not revert the inhibiting action 2,4 dinitrophenol, arsenite, urethane, p-Cl Hg benzoate, iodoacetate and o-iodosobenzoate. In controls made with ATP only no granule extrusion was observed.

In order to verify if the action of 48–80 is due to a disruption of cell membranes a probable hemolytic activity of this Compound was assayed on washed sheep erythrocytes, following Kracke and Parker's (1940) instructions. No hemolysis occurred in concentrations of 1/50,000 to 1/2,000.

When observed under phase microscopy the action of Compound 48–80 in living mesentery spreads was characterized by the rapid formation of intracellular vacuoles, swelling of the cells and extrusion of the granules. As far as could be observed, no disruption of the cells occurred, and the cell cytoplasm was apparently maintained. This sequence of phenomena was well observed in serial photographs of the same cell before and after the action of 48–80 (Fig. 1).

*Discussion.* From the analysis of the results obtained, we would like to stress the extremely high activity of Compound 48–80, and that its action is very probably dependent on chemi-

cal phenomena related to the mast-cell metabolism. The fact that temperatures below 25° C., a low pH, and several metabolic inhibitors, inhibit the extrusion of the mast-cell granules consubstantiates this view. It is interesting to observe that all —SH blocking compounds (p-Cl Hg benzoate, o-iodosobenzoate, iodoacetate) used, inhibited the action of Compound 48–80, and that the inhibition is reverted by the addition of reduced glutathione. This fact highly suggests an active participation of the —SH groups present in structural proteins or enzymes, in the extrusion of mast-cell granules promoted by Compound 48–80.

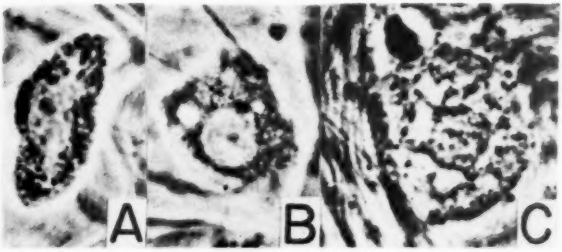


FIG. I. Mast-cells of rat mesentery before (A) and after the action of Compound 48–80 (B and C). Observe cytoplasmic vacuolisation and extrusion of granules. Phase contrast of living mesentery spread.

The fact that 2,4 dinitrophenol, arsenite and urethane inhibit the action of Compound 48–80 suggest that phosphorilative processes are involved in these phenomena; one should however state that after adding ATP this inhibition could not be reverted. That other metabolic phenomena might also be involved in the responses of mast-cells to Compound 48–80, is also suggested by the inhibiting action observed after the addition of cyanide. It is difficult to verify if Compound 48–80 acts on the cell membrane, cytoplasm or in both.

The fact that uranil nitrate, a metabolic inhibitor, described as acting on the cell membrane enzymes (Barron, Muntz, and Gasvoda, 1948) inhibits granule extrusion induced by the addition of Compound 48–80, suggests the participation of the cell membrane on the processes studied.

On the other side, the inhibition promoted by cyanide due to its action on cytochrome oxydase system, present as we know,

to a great extent in the mitochondria (Schneider, 1946; Hogeboom, Claude, and Hotchkiss, 1946; Schneider, Claude, and Hogeboom, 1948) suggests its activity in the cytoplasm.

A comparison between the results obtained applying a similar analysis to different cell types will probably lead to a better understanding of the processes related to cell function.

*Summary.* The action of Compound 48–80, at low concentrations, promoting a vigorous extrusion of mast-cell granules, was studied.

It was observed that low temperature, acid pH and several metabolic inhibitors, prevent granule extrusion observable after the addition of Compound 48–80.

Glutathione reverts the action of the so-called sulfhydrile blocking substances (iodoacetate, o-iodosobenzoate and p-Cl Hg benzoate) while ATP does not revert the action of 2,4 dinitrophenol, arsenite and urethane.

It is suggested that metabolic phenomena occurring in mast-cells are of importance in their response to the action of Compound 48–80.

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# EFFECT OF ESTROGEN ON ANTERIOR PITUITARY CYTOPHYSIOLOGY*

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Recent improvements in technical methods have led to changes in our understanding of basic cellular physiology of the anterior pituitary gland and have made it desirable to reinvestigate the effects of the estrogenic hormones on pituitary structure and function.

Since first described by Addison (1917), gonadectomy has been known to cause dramatic increase in size and relative number of pituitary basophile cells. Simultaneous increase in gonadotrophic hormone content of the hypophysis has suggested that these cells are the source and storage place of at least one of the gonadotrophic hormones, FSH; while conflicting opinions are held as to whether they, or the acidophiles, produce LH. Conversely, the percentage of basophile cells is reduced when estrogen is administered to gonadectomized animals, the amount of response being dependent upon the dosage and duration of treatment (Baker and Everett, 1944; Finerty and Meyer, 1950).

These observations fit very well the concept of a reciprocal regulation of gonadotrophic secretion of the anterior pituitary by gonadal hormone levels in the blood. Meyer, Biddulph, and Finerty (1946) on the basis of their studies of estrogen-treated parabiotic rats postulated that: (1) FSH is produced by the basophiles and LH by the acidophiles of the pituitary gland, (2) differentiation of each type of chromophile from chromophobes is controlled by the level of blood estrogen, and (3) the rate at which the chromophiles release their gonadotrophin is dependent upon the level of blood FSH and LH.

The experiments described in this paper were designed to correlate such advances as the concept of "gonadotroph" and "thyrotroph" types of basophiles with the previous studies in which basophiles were classified as one cell type, and to add

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further evidence to support the differentiation of this cell type as advocated by Halmi (1950) and by Purves and Griesbach (1951).

So that the conclusions in this presentation may be more easily understood, a summary of the presently accepted nomenclature for the cell types and secretory products of the rat pituitary gland as interpreted by the present authors is included.

| *Cell Types* | *Secretory Products* |
|---|---|
| acidophile | growth hormone, lactogenic hormone, ACTH, LH (?) |
| beta basophile (thyrotroph) | thyrotrophic hormone (TSH) |
| delta basophile (gonadotroph) | gonadotrophic hormone FSH, LH (?) |
| chromophobe | none |

*Methods.* Female rats obtained from the Holtzman Rat Company were used in this experiment. All were born on the same day and were shipped at weaning. At the age of 25 days, 66 rats were ovariectomized. On each of the first, second, fourth, eighth, sixteenth, and thirty-second post-operative days, six of the ovariectomized rats were autopsied. The remaining thirty were injected daily, starting on the sixteenth post-operative day, with 0.1 microgram of estradiol (subcut.). Likewise six of these were sacrificed on each of the first, second, fourth, eighth and sixteenth days following beginning of the injections. Intact control rats (6 each day) were autopsied at 25 days, 41 days and 57 days of age.

At the time of autopsy, the body weight and the uterine weight of each animal were recorded and the pituitaries were placed in fixing solution. For each group of six animals, two were fixed in Zenker-formol, two in formol-sublimate, and two in Susa-picric acid. Those fixed in Zenker-formol were stained by the procedure of Briseno-Castreion and Finerty (1949), after which the acidophiles were a purplish-red and the basophiles a light green color. PAS (McManus, 1946) was used to stain the pituitaries fixed in formol-sublimate and the basophiles appeared a purplish-red and the acidophiles a light blue. When Susa-picric acid was used as a fixative, Halmi's staining technique (1952) was used and the beta cells contained granules that were purple and the delta cells contained granules that were a grayish-green.

The twenty-two pituitaries stained with azocarmine were studied to determine the effect of ovariectomy and injections of estradiol on the percentage of the various cell types. For each pituitary one section as near the middle as possible was analyzed for percentage of cell types.

Central sections were chosen for counting because it was realized that it would be easier to approximate the same region in different pituitaries if the posterior and intermediate lobes could be used as landmarks and possibly that the counts would be more accurate if made in larger sections. However, Finerty and Meyer (1950) had found that there was no significant difference in the percentage when they counted three horizontal sections at equidistant levels in each gland or when a single central section was counted.

In every tenth field of each such section the number of acidophiles, basophiles and total number of cells were counted at a magnification of 1,000 X. The percentage of basophiles and acidophiles were then calculated.

In the second part of this work a study was made of the number of basophiles demonstrated when stained with the PAS method. Three sections of one pituitary in each group were studied. Every basophile was counted under oil immersion in the complete section, and the number of basophiles in each field was counted and placed in its relative position in an outline diagram of the section. Then the total number of basophiles in the section was calculated. As each section was studied, particular attention was paid to the size of the basophiles and their cytological appearance in relation to granules, staining intensity and negative-Golgi images. Then the diagrams showing the regional distribution of the basophiles were studied.

The glands stained with Halmi's stain were studied in a manner similar to that used in studying the PAS stained pituitaries. All the thyrotrophs were counted under high power in three complete sections of one pituitary of each group, and their location in the anterior lobe and their number were noted.

## *Results*

The body weights progressively increased with age from an average 53 gms. for those autopsied at 25 days of age to 84 gms. for those autopsied at 33 days of age and having been ovariectomized for eight days. The intact controls autopsied at 41 days of age had an average body weight of 112 gms. whereas, the ovariectomized rats autopsied at the same time averaged 131 gms. in weight. Intact controls autopsied at 57 days of age averaged 161 gms., while the ovariectomized animals with 16 injections autopsied at the same time averaged 145 gms., and the ovariectomized with no injections averaged 189 gms. Ovariectomy caused an increase in weight and the injections decreased the body weight as demonstrated in table 1.

The uterine weights of the ovariectomized rats up to 41 days of age, as shown in the same table, varied little from those of the intact controls autopsied at 25 days of age which had an average of 30.9 mgms. The intact controls autopsied at 41 days had an average uterine weight of 99.0 mgms., which was more than three times the weight of ovariectomized animals of the same age. After one injection of estradiol a distinct difference in uterine weight was noticeable over that of the ovariectomized animals. Uterine weight continued to increase with the number of injections until 57 days of age, at which time it approached that of the 57 day old intact controls. The castrate animals with

no estrogen injections had an average uterine weight of 25.7 mgms., one-ninth that of the intact controls of the same age.

TABLE 1

*Effect of Ovariectomy and Estrogen Treatment on Body Weight and Uterine Weight of Young Female Rats*

| Treatment | No. of Rats | Age (days) | Body Wt. (gm.) | Uterine Wt. (mgm.) |
|---|---|---|---|---|
| Intact controls ..... | 6 | 25 | 53 | 30.9 |
| Ovariectomized and autopsied 1 day later ..... | 6 | 26 | 56 | 23.8 |
| Ovariectomized and autopsied 2 days later ..... | 6 | 27 | 58 | 19.9 |
| Ovariectomized and autopsied 4 days later ..... | 6 | 29 | 65 | 23.8 |
| Ovariectomized and autopsied 8 days later ..... | 6 | 33 | 84 | 23.3 |
| Ovariectomized and autopsied 16 days later ..... | 6 | 41 | 131 | 21.4 |
| Intact controls ..... | 6 | 41 | 112 | 99.0 |
| Ovariectomized 1 injection ..... | 6 | 42 | 130 | 43.0 |
| Ovariectomized 2 injections ..... | 6 | 43 | 116 | 93.8 |
| Ovariectomized 4 injections ..... | 6 | 45 | 127 | 136.0 |
| Ovariectomized 8 injections ..... | 6 | 49 | 132 | 175.8 |
| Ovariectomized 16 injections ..... | 6 | 57 | 145 | 207.5 |
| Ovariectomized no injection ..... | 6 | 57 | 189 | 25.7 |
| Intact controls ..... | 8 | 57 | 161 | 234.5 |

In studying the pituitary glands stained with azocarmine it was found that the mean percentage of acidophiles varied within a range of 30 per cent to 39 per cent in all cases except three. The intact controls at 25 days of age averaged 28 per cent. In those rats which were ovariectomized for 16 days and had one injection of estradiol (42 days of age) the percentage reached 45 per cent. Also, in those rats which were ovariectomized for 32 days and received no injections, the average was 44 per cent.

The changes in the percentage of basophiles were easily correlated with the different conditions of treatment and are shown on table 2. The mean percentage of basophiles in the intact controls and in the ovariectomized rats up to 4 days after ovariectomy showed no significant changes with a variation of from 3 to 5 per cent. However, on the eighth day after ovariectomy the total basophiles had increased to 7 per cent and on the sixteenth day following ovariectomy they had reached 11 per cent while the intact controls autopsied at the same time had an average of 6 per cent basophiles.

The injections were begun 17 days after ovariectomy and after two injections a slight decrease in basophile percentage was noticeable. After sixteen daily injections 4 per cent basophiles were present, the intact controls autopsied at the same age averaged 5 per cent while the castrate animals having had no injections showed 13 per cent basophiles. The size of the basophiles began to increase following ovariectomy and many exhibited the hypertrophied negative Golgi images as early as 4 days after operation.

Table 3 shows the changes in the average number of basophiles per field as found when studying the pituitaries stained with PAS in relation to the time and treatment. It was realized that total numbers were difficult to interpret due to variation in size of the pituitaries and to the difficulty of selecting exactly corresponding sections in all glands. Therefore the total number of basophiles per section was divided by the number of fields per section so that comparison could be made of mean number of basophiles per field.

The basophiles in the 25-day-old intact controls were relatively hard to distinguish. In no cells was a negative Golgi image found, however, some cells seemed to have a clear area around the nucleus. The basophiles were very small in relation to those found in the ovariectomized animals. Little difference was noticed at one or two days after ovariectomy. By four days following the operation, the basophiles were becoming larger with increasing numbers of negative Golgi bodies. Sixteen days following ovariectomy the basophiles were quite large, oval and many negative Golgi bodies were noticeable. In the intact con-

TABLE 2

*Effect of Ovariectomy and Estrogen Treatment on Pituitary Cell Type Analysis (Azocarmine Stain)*

| Rat No. | Treatment | Age at Autopsy (days) | Pituitary Cell Type Analysis Per cent Acid | Per cent Baso. | Per cent Chromo. |
|---|---|---|---|---|---|
| L1 | Intact control ..... | 25 | 27 | 6 | 67 |
| L2 | Intact control ..... | 25 | 29 | 5 | 66 |
| L7 | Ovariectomized and autopsied 1 day later ..... | 26 | 30 | 3 | 67 |
| L8 | " .... | 26 | 29 | 3 | 68 |
| L13 | Ovariectomized and autopsied 2 days later ..... | 27 | 37 | 3 | 60 |
| L14 | " .... | 27 | 36 | 3 | 61 |
| L19 | Ovariectomized and autopsied 4 days later ..... | 29 | 33 | 3 | 64 |
| L20 | " .... | 29 | 31 | 5 | 64 |
| L25 | Ovariectomized and autopsied 8 days later ..... | 33 | 39 | 7 | 54 |
| L26 | " .... | 33 | 39 | 7 | 54 |
| L31 | Ovariectomized and autopsied 16 days later ..... | 41 | 43 | 11 | 46 |
| L32 | " .... | 41 | 31 | 11 | 58 |
| L37 | Intact controls .... | 41 | 33 | 7 | 60 |
| L38 | " .... | 41 | 30 | 6 | 64 |
| L43 | Ovariectomized 1 injection ..... | 42 | 45 | 10 | 45 |
| L44 | " .... | 42 | 46 | 15 | 39 |
| L49 | Ovariectomized 2 injections ..... | 43 | 38 | 8 | 54 |
| L50 | " .... | 43 | 40 | 9 | 51 |
| L55 | Ovariectomized 4 injections ..... | 45 | 42 | 10 | 48 |
| L56 | " .... | 45 | 35 | 10 | 55 |
| L61 | Ovariectomized 8 injections ..... | 49 | 36 | 6 | 58 |
| L62 | " .... | 49 | 38 | 12 | 50 |
| L67 | Ovariectomized 16 injections ..... | 57 | 38 | 3 | 59 |
| L68 | " .... | 57 | 40 | 4 | 56 |
| L73 | Ovariectomized no injections ..... | 57 | 44 | 13 | 43 |
| L74 | " .... | 57 | 45 | 14 | 41 |
| L79 | Intact controls .... | 57 | 37 | 4 | 59 |
| L80 | " .... | 57 | 28 | 3 | 69 |
| L85 | " .... | 57 | 39 | 7 | 54 |
| L86 | " .... | 57 | 38 | 6 | 56 |

TABLE 3

*Effect of Ovariectomy and Estrogen Treatment on Pituitary Basophile Cells (PAS Stain)*

| Rat No. | Treatment | Age (days) | Av. no. of baso. per field | Av. no. of baso. per field per pit. |
|---|---|---|---|---|
| L3 | Intact control ..... | 25 | 6.0<br>6.0<br>3.8<br>4.0 | 4.9 |
| L9 | Autopsy 1 day after ovariectomy ... | 26 | 3.0<br>4.0<br>4.6<br>3.9 | 3.9 |
| L16 | Autopsy 2 days after ovariectomy ... | 27 | 4.1<br>4.5<br>5.6 | 4.7 |
| L21 | Autopsy 4 days after ovariectomy ... | 29 | 9.2<br>10.6<br>9.1 | 9.6 |
| L28 | Autopsy 8 days after ovariectomy ... | 33 | 12.4<br>12.0<br>14.4 | 12.9 |
| L33 | Autopsy 16 days after ovariectomy ... | 41 | 17.9<br>19.4<br>21.3 | 19.5 |
| L40 | Intact control ..... | 41 | 3.9<br>3.6<br>3.6 | 3.7 |
| L45 | One injection ..... | 42 | 18.3<br>18.0<br>19.3 | 18.5 |
| L51 | Two injections ..... | 43 | 13.5<br>13.9<br>13.7 | 13.7 |
| L57<br>L58 | Four injections ..... | 45 | 12.2<br>13.2<br>13.6<br>13.2 | 13.0 |
| L64 | Eight injections ..... | 49 | 9.8<br>9.5<br>10.1 | 9.8 |
| L70 | Sixteen injections ... | 57 | 4.6<br>4.8<br>4.9 | 4.8 |
| L76 | Autopsy 32 days after ovariectomy ... | 57 | 17.8<br>20.0<br>16.3 | 18.0 |
| L82 | Intact controls ..... | 57 | 4.1<br>3.8<br>4.1 | 4.0 |

trols at 41 days of age a picture similar to that at 25 days was seen.

The castration changes in the pituitary were gradually reversed by estradiol administration and after 16 injections the hypertrophied basophiles were relatively scarce and confined to the area adjacent to the intermediate lobe. By this time the basophiles had become small and greatly decreased in number. They were quite hard to distinguish and were pale. In the ovariectomized animals killed at the same time (57 days of age) the basophiles were extremely large and all had a negative Golgi image whereas the intact controls resembled the conditions found in the younger controls.

The diagrams of the pituitaries were then studied to determine where in the anterior lobe the greatest increase in numbers occurred. In the 25-day-old intact controls they seemed to be more localized at the periphery. In many regions they appeared to be lined up along the edges. In the animals ovariectomized for one day this peripheral localization still persisted. Two days following ovariectomy this was still noted. However the central fields seemed to contain more than they had previously as there was only one central field in the entire section that contained no basophiles whereas four to six fields usually had been seen before. Also it was noted in this section that the peripheral basophiles were more granular and darker staining whereas some of the central ones appeared to have lost their cytoplasm.

Four days following ovariectomy the number of basophiles had increased in the peripheral fields and were definitely more darkly stained than the central ones. Many in the center appeared degranulated or degenerating. As many as twenty or twenty-two appeared in some fields in central regions, which was more than were found even in the peripheral regions previously.

In the animals ovariectomized for eight days, almost all regions contained more than were found at shorter post-castration periods. At this time, it seemed that they were more evenly distributed than before. The ventral portion of the anterior lobe, however, still showed the greatest concentration of basophiles. By sixteen days following ovariectomy this ventral localization of basophiles was marked by an even more pro-

nounced central concentration. All fields had increased in number over those of the earlier stages, but there was no doubt left that the central regions contained many more than the peripheral ones. They also appeared to be in almost solid masses adjacent to the intermediate lobe. A similar picture of the darker staining ones at the periphery was seen. The intact controls at 41 days of age were similar to the twenty-five day old intact controls with peripheral localization.

In the ovariectomized animal with one injection, it appeared that there were fields with greater numbers of basophiles along the edge in parts of the anterior lobe. However, in many central regions approximately the same numbers appeared as in the peripheral fields and looking at the picture as a whole central localization was still dominant. Darker staining ones still appeared along the edges. After two injections the number found in the central fields was greatly decreased. In the previous one many central fields contained over eighteen basophiles, but in this one no such number was found.

By the fourth injection, it was easily seen that the greatest numbers were found peripherally. However, the central and peripheral fields still contained more than the corresponding regions of the intact controls.

After eight injections the peripheral fields contained only half as many as were found four days previously, but still greater numbers were found in the periphery than in central regions. By sixteen injections the picture was similar to that of the 57-day-old intact controls (Fig. I) with many central regions con-

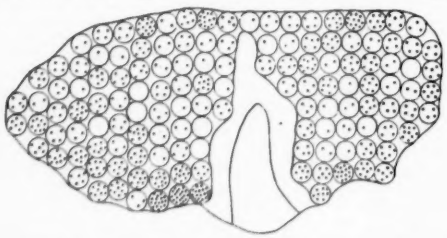

FIG. I. Diagrammatic representation of the distribution of basophiles in a section of the anterior hypophysis of an intact 57-day-old female rat. Each circle represents a microscopic field and the enclosed dots represent the number of basophile cells (beta and delta type) in that field. The sketch represents an actual count of a PAS-stained gland, sectioned at 4 micra. Note the peripheral concentration, especially adjacent to the pars intermedia.

taining no basophiles and a tremendous decrease in both peripheral and central fields over those of the 57-day-old ovariectomized animal in which there was a clear picture of the greatest numbers being located centrally (Fig. II).

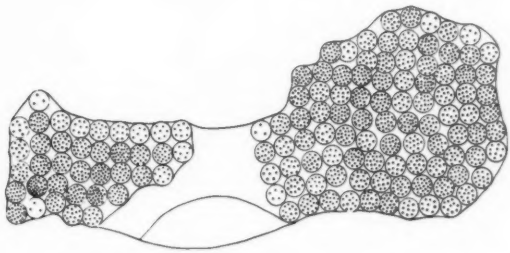

FIG. II. Diagrammatic representation of the distribution of basophiles in a section of the anterior hypophysis of a female rat which had been ovariectomized 32 days previously. Note increase in number and relatively even distribution of basophile cells, which are predominantly of the delta, or gonadotroph, type.

The pituitary glands stained with Halmi's stain were then studied, and it was found that no obvious differences could be demonstrated in thyrotrophs after ovariectomy or after the injections of estradiol as related to number and localization in the anterior lobe. This is demonstrated in table 4. However, it was clearly seen that the central localization of thyrotrophs did occur in all the pituitaries, with many of the peripheral fields containing none.

It follows therefore that the marked changes in the total number of basophiles as seen with the PAS method can be only a reflection of a differential increase in delta type basophiles or gonadotrophs.

*Discussion.* Morphological features and regional localization of thyrotrophs and gonadotrophs of the anterior pituitary gland described by Purves and Griesbach (1951) have been confirmed in this study. The delta type of basophile (Halmi, 1950), or gonadotroph, was shown to be the cell type which is most significantly affected by ovariectomy during a 32-day period, or by ovariectomy for 16 days followed by replacement therapy with estradiol. Increase in percentage of acidophiles after 1 injection of estradiol also may support the contention of Finerty

TABLE 4

*Effect of Ovariectomy and Estrogen Treatment on Beta Cell Counts (Halmi's Stain)*

| Rat No. | Treatment | Age (days) | Av. no. of beta cells per field | Av. no. of beta cells per field per pit. |
|---|---|---|---|---|
| L6 | Intact control ..... | 25 | 7.9<br>7.3<br>3.5<br>4.9 | 5.9 |
| L11 | Autopsy 1 day after ovariectomy ... | 26 | 4.2<br>6.3<br>7.2 | 5.9 |
| L17 | Autopsy 2 days after ovariectomy ... | 27 | 4.5<br>5.2<br>7.4 | 5.7 |
| L24 | Autopsy 4 days after ovariectomy ... | 29 | 3.7<br>5.4<br>5.3 | 4.8 |
| L29 | Autopsy 8 days after ovariectomy ... | 33 | 7.4<br>4.9<br>6.6 | 6.3 |
| L36 | Autopsy 16 days after ovariectomy ... | 41 | 5.2<br>9.5<br>2.3 | 5.7 |
| L41 | Intact controls ..... | 41 | 7.3<br>6.0<br>8.3 | 7.2 |
| L47 | Ovariectomized 1 injection ..... | 42 | 3.9<br>4.6<br>4.9 | 4.5 |
| L53 | Ovariectomized 2 injections ..... | 43 | 4.0<br>4.5<br>5.1 | 4.5 |
| L60 | Ovariectomized 4 injections ..... | 45 | 3.3<br>3.0<br>2.3 | 2.9 |
| L65 | Ovariectomized 8 injections ..... | 49 | 2.8<br>5.9<br>4.7 | 4.5 |
| L72 | Ovariectomized 16 injections ..... | 57 | 5.4<br>3.6<br>4.2 | 4.4 |
| L78 | Autopsy 32 days after ovariectomy ... | 57 | 4.1<br>4.4<br>4.5 | 4.3 |
| L84 | Intact controls ..... | 57 | 7.0<br>7.0<br>4.0 | 6.0 |

and Meyer (1950) that low doses of estrogen or short periods of injection, increase the number of acidophilic cells.

Gonadotrophs were especially prominent along the superior surface of the anterior lobe adjacent to the intermediate lobe and on the periphery of the gland as well as on the entire inferior surface. Increase in size and number of gonadotrophs and a shift in this localization were noted following ovariectomy. The change in position occurred gradually with a spread from peripheral predominance of gonadotrophs to a state in which more were present centrally. However, it appears that this is not a migration at the expense of peripheral basophiles since even peripheral zones increased greatly in numbers. It is suggested that estrogen deprivation induces an increase in number of gonadotrophs by removal of inhibition of formation of chromophobes to gonadotrophic type basophiles, especially in central areas.

Observations of beta type basophiles, or thyrotrophs (Halmi, 1950), supported previous studies in showing primary central localization. No significant change in number, size or position of thyrotrophs was noted after either ovariectomy or estrogen treatment.

Frequent observations in this work of differences in staining capacity and granulation of more peripherally located gonadotrophs may support the suggestions of Zeckwer (1937), Purves and Griesbach (1952) and Farquhar and Rinehart (1954) that there are two morphologically different types of gonadotrophs. Rapid decrease in number of centrally located gonadotrophs following estrogen injection at a time corresponding to rapid "release" of LH is also suggestive. It is conceivable that such a finding might rationalize current disagreements as to the source of LH. If LH can be shown to be produced by a form of gonadotroph, the previous observation of acidophilic cell changes after estrogen may possibly be attributed to stimulation of ACTH activity.

The data presented in this paper confirm the existence of two morphologically and functionally different types of basophile cells, the "gonadotroph" and "thyrotroph," and add support to the suggestions that two gonadotrophs may be present. These findings encourage the hope of pituitary cytophysiologists that

sometime each anterior pituitary hormone can be assigned to a specific cell type.

*Summary*

Young female rats were ovariectomized and after 16 days were injected with 0.1 microgram of estradiol, daily, for an additional 16 days. Ovariectomized, uninjected, and intact female rats of the same age served as controls. The dosage of estradiol used returned the increased percentage of pituitary basophile cells and atrophied uteri to normal. Ovariectomy resulted in increased body weight gain, whereas estrogen caused growth retardation. Histological studies of the anterior pituitary gland were made using an azocarmine stain for differential cell counts, PAS stain for basophile cell localization, and aldehyde-fuchsin stain for differentiation of "thyrotroph" cells. The existence of two types of basophiles and their localization; "gonadotrophs" adjacent to intermediate lobe, on periphery and ventral surface of anterior lobe; "thyrotrophs" centrally placed; were confirmed. Ovariectomy resulted in increased number of "gonadotrophs" throughout anterior lobe; estrogen therapy reversed the condition. Thyrotrophs were not affected by estrogen deprivation or replacement.

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# UTILIZATION OF HYDROCORTISONE IN ARTHROTOMY OF THE KNEE*

S. F. Moorehead

This review covers the post-operative intra-articular utilization of hydrocortisone in knee arthrotomies performed for excision of torn semilunar cartilages at Lackland Air Force Base from March 1953 through March 19, 1954. Our interest lay in an attempt to find a method of treatment which would return an airman to duty more rapidly. One of our civilian consultants, Doctor John Hinchey, suggested using Compound "F" post-operatively in these arthrotomies. He had used the hormone in a limited number of his own cases and had obtained excellent results. He was impressed by the increased speed of recovery and by the decreased amount of post-operative pain and swelling.

No detailed reference could be found reporting the utilization of hydrocortisone post-operatively in arthrotomies of the knee. However, Hallander and associates reported the value of injections of this hormone in osteoarthritic joints. Compound "F" differs from cortisone only in a single structural detail, that being the presence of a hydroxyl radical instead of a ketone group at the C-11 position of the steroid nucleus. Compound "F" has a longer duration of action than cortisone when placed in osteoarthritic joints. This is explained by its being less soluble in blood plasma. Therefore, its local effect is enhanced by slower dispersion from the joint. The exact method of its reaction in a joint is not known. However its beneficial clinical action is borne out by its effectiveness in a variety of inflammatory joint diseases. No cure has been established by Compound "F". Its value, according to Boland, lies in its ability to temporarily alleviate symptomatology by its local action.

In our series, a total of fifty-four arthrotomies were performed on fifty-three patients. All were diagnosed as meniscus tears ex-

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cept six, which were cysts of the lateral meniscus. Of the remaining 48, there were six lateral, thirty-five medial meniscii and seven negative explorations. In the seven arthrotomies in which no semilunar cartilage tear was found, two showed no joint pathology; two had chondromalacia of the patella sufficient in degree to require excision of the area involved, and the other three had a partial, acute tear of the anterior cruciate ligament with resultant limitation of extension. Whether the cruciate tear limited full extension or was associated with collateral ligamentous damage, is not known. However, by clinical examination, the collateral ligaments and anterior cruciate ligaments were intact.

Our surgical approach to the knee joint was a short anterior incision, approximately 5 to 7 cm. in length. If necessary, a separate posterior incision was made to remove the cartilage or a posterior cartilaginous tab. Initially, the post-operative care was planned to alternate traction with immobilization in a plaster cylinder. This standardization of post-operative management was not accomplished. Traction was used in twelve cases and plaster casts were used to immobilize the remaining forty-one cases. The shortest period of immobilization was three days, the longest, eighteen days, with a total average of 6.6 days. No conclusions could be drawn from this review as to which method of treatment was more desirable. No ill effects from immobilization were noted.

The average total length of hospitalization of each patient was 45.5 days. There was no significant difference in the length of hospitalization of those patients in which Compound "F" was used and in those that did not receive the hormone. The prerequisite for an airman to be discharged from the hospital following an arthrotomy of his knee was quadriceps strength sufficient to lift 30 lbs. ten times through a full range of motion. He should walk with a normal gait, have minimal or no effusion of the joint, and have a functional range of motion with full extension.

The longest period of hospitalization was 118 days. In this case no specific pathology was demonstrated. However, a partial lipectomy of the pre-patellar fat pad was performed and three incisions were utilized to explore the joint. In addition, this patient had a severe post-operative penicillin reaction. The shortest peri-

od in the hospital was fifteen days. The latter was the first patient treated with hydrocortisone post-operatively.

The hormone was injected intra-articularly in thirty knees prior to closing the operative wound. The remaining twenty-four arthrotomies were used as controls. In general, Compound "F" was used in alternate cases without selection.

The criteria used to judge our results in utilizing Compound "F" consisted of: (1) the amount of post-operative narcotic required (2) the post-operative effusion (3) the period of time required to perform straight leg raising and quadriceps contraction (4) the date of ambulation (5) the date of discharge from the hospital.

At first only 25 mg. of hydrocortisone was used immediately post-operatively and no benefit was noted. The amount was then increased to 50 mg. and subsequently to 75 mg. prior to closing the operative wound. This did not produce any earlier restoration of function, when judged by the standard criteria.

*Summary*

Arthrotomies performed for semilunar cartilaginous tears have been reviewed with reference to the utilization of intra-articular Compound "F". It is concluded that hydrocortisone is of no value in improving the immediate post-operative course following semilunar cartilage excision.

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# MAMMAL FLEAS OF SANTA FE COUNTY, NEW MEXICO*

HARVEY B. MORLAN

The biological relationships of mammals, ectoparasites, and sylvatic plague were the primary subjects of a field study established at Santa Fe by the Communicable Disease Center in July, 1951. A general description of the study has been prepared by Holdenried and Morlan (manuscript). The present report lists the species of fleas found on mammals or in their nests together with a summary of available information on host relationships, sex ratio, local distribution, and seasonal abundance.

From July, 1951, to February, 1954, over 26,000 fleas representative of 51 species were collected from the 8,784 mammals examined (and/or re-examined) for ectoparasites. Collections from rodent nests added two additional species of fleas for a total of 53 species presently known to be associated with 36 species of Santa Fe County mammals. Comprehensive seasonal data were obtained for a two year period, October, 1951, through September, 1953, by intensive weekly collections at the Santa Fe Municipal Airport.

*Mammals.* Mammals are listed in the order given by Simpson (1945). Common names and subspecific designations are shown only in the following list which provides a compact mammal-flea index; numbers following the name of each species of mammal refer to the numbered species of fleas.

Dusky shrew, *Sorex obscurus obscurus* Merriam—(6 examined, not infested).

Western big-eared bat, *Corynorhinus rafinesquii pallescens* Miller—(6 examined, not infested).

Pika, *Ochotona princeps incana* Howell—14, 21, 24, 26, 33.

Blacktail jackrabbit, *Lepus californicus texianus* Waterhouse—1, 2, 35.

Desert cottontail, *Sylvilagus audubonii warreni* Nelson—1, 2, 11, 13, 25, 37, 43.

Spruce squirrel, *Tamiasciurus hudsonicus mogollonensis* (Mearns)—21, 34, 36.

Yellowbelly marmot, *Marmota flaviventris obscura* Howell—47.

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* From the Communicable Disease Center, Public Health Service, U.S. Department of Health, Education, and Welfare, Atlanta, Ga. Received for publication November 3, 1954.

Whitetail prairie dog, *Cynomys gunnisoni zuniensis* Hollister—3, 4, 13, 38, 39, 47.

Spotted ground squirrel, *Citellus spilosoma marginatus* Bailey—3, 4, 10, 11, 12, 13, 37, 41, 48.

Rock squirrel, *Citellus variegatus grammurus* (Say)—3, 4, 13, 27, 38, 41.

Golden-mantled squirrel, *Citellus lateralis lateralis* (Say)—27, 34, 44, 47.

Least chipmunk, *Eutamias minimus operarius* Merriam—34.

Colorado chipmunk, *Eutamias quadrivittatus quadrivittatus* (Say)—6, 22, 34, 37, 44, 52.

Northern pocket gopher, *Thomomys talpoides fossor* Allen—18, 20, 24, 28, 34.

Valley pocket gopher, *Thomomys bottae pervagus* Merriam—28.

Silky pocket mouse, *Perognathus flavus flavus* Baird—2, 9, 10, 11, 12, 13, 18, 41.

Bannertail kangaroo rat, *Dipodomys spectabilis baileyi* Goldman—2, 10, 11, 12, 13, 16, 35, 43, 46, 48.

Ord kangaroo rat, *Dipodomys ordii medius* Setzer—11, 12, 13, 16, 35, 37, 41, 46, 48, 52.

Western harvest mouse, *Reithrodontomys megalotis aztecus* Allen—11, 13, 27, 37, 41, 48, 50, 52.

Deer mouse, *Peromyscus maniculatus rufinus* (Merriam)—7, 9, 11, 12, 13, 15, 19, 21, 29, 31, 32, 34, 37, 41, 43, 46, 48, 50, 52.

White-footed mouse, *Peromyscus leucopus tornillo* Mearns—4, 9, 18, 21, 31, 35, 37, 41, 46, 48, 50, 52.

Brush mouse, *Peromyscus boylii rowleyi* (Allen)—3, 5, 31, 37, 41.

Piñon mouse, *Peromyscus truei truei* (Shufeldt)—2, 5, 9, 10, 11, 13, 18, 19, 20, 27, 29, 31, 37, 41, 43, 46, 50, 52.

Rock mouse, *Peromyscus nasutus nasutus* (Allen)—5, 9, 15, 20, 29, 31, 36, 37, 52.

Northern grasshopper mouse, *Onychomys leucogaster pallescens* Merriam—9, 10, 11, 12, 13, 16, 31, 35, 37, 41, 43, 46, 48.

Southern plains woodrat, *Neotoma micropus canescens* Allen—9, 11, 12, 13, 16, 18, 30, 35, 37, 41, 42, 43, 46, 48, 50.

Whitethroat woodrat, *Neotoma albigula albigula* Hartley—3, 4, 5, 9, 10, 11, 12, 13, 16, 18, 20, 21, 22, 23, 27, 31, 35, 37, 41, 42, 43, 46, 48.

Mexican woodrat, *Neotoma mexicana fallax* Merriam—18, 22, 31, 34, 35, 42, 43.

Bushytail woodrat, *Neotoma cinerea orolestes* Merriam—6, 7, 22, 37, 42, 43.

Boreal redback vole, *Clethrionomys gapperi guati* Cockrum and Fitch—17, 29.

Longtail vole, *Microtus longicaudus mordax* (Merriam)—6, 7, 8, 32, 33, 34, 37, 51, 53.

Western jumping mouse, *Zapus princeps princeps* Allen—(2 examined, not infested).

Longtail weasel, *Mustela frenata nevadensis* Hall—(1 examined, not infested).

Longtail weasel, *Mustela frenata neomexicana* (Barber and Cockerell)—(2 examined, not infested).

Striped skunk, *Mephitis* sp.—4.
Spotted skunk, *Spilogale* sp.—3, 18, 27.

*Mammal Nests.* Very little time was available for a study of nest fauna. From October 1951 through November 1953, 66 rodent nests were collected in Santa Fe County. Arthropods were separated from other material by use of modified Berlese funnels. Twenty-eight nests of *Neotoma albigula* were collected, one or more in each month of the year. Nine *Perognathus flavus* nests were taken in each month from November through April and one in August. Six nests of *Dipodomys spectabilis* were collected in January, February, April, and May. Two *Dipodomys ordii* nests were taken in February and May. Five nests of *Peromyscus truei* were collected in each month from March through June. A single *Peromyscus leucopus* nest was obtained in March. Four nests of *Tamiasciurus hudsonicus* were collected in October. A single *Microtus longicaudus* nest was taken in September. Nests of *T. hudsonicus* were from the Canadian Zone; the *M. longicaudus* nest was from the Transition Zone; all other nests were collected in the Upper Sonoran Zone.

*Fleas.* Beginning in January, 1952, most of the female fleas were examined under the dissecting microscope to determine the extent of oöcyte development. When oöcytes were obscured by heavy engorgement or an especially dark integument, the abdomen was either manipulated or dissected with teasing needles to permit accurate observation. A female was considered to be gravid if the larger oöcytes were at least half the size of a mature egg. The number of females examined and the number classed as gravid were recorded by months. The data have been summarized separately for each species of flea.

All blood-sucking arthropods are potential vectors of disease. Knowledge of important transmitters of sylvatic plague in nature remains fragmentary. Macchiavello (1954) has published the first in a series of papers to review the reservoirs and vectors of plague; fleas that occur in Santa Fe County and are included in his list of plague vectors have been indicated by an asterisk (*).

Arrangement of flea species follows Holland (1949) and Jellison *et al.* (1953). Under each species of flea, associated host animals are listed in phylogenetic order followed by: (1) the

number of animals examined; (2) percentages of animals infested; (3) mean number of fleas per animal examined; (4) mean number of fleas per infested animal. Percentages were computed to the nearest whole number and means to the nearest tenth. Percentages of less than one or means of less than one-tenth are followed by a minus sign (—).

1. *Cediopsylla inaequalis inaequalis* (Baker)

Local distribution: Most records were from the Santa Fe Airport; a few from other areas in the Upper Sonoran Zone. Six undetermined specimens of *Sylvilagus* from the Transition Zone were also infested.

Host records:

| | | | | |
|---|---|---|---|---|
| *Lepus californicus* ..... | 4; | 25 ; | 1.2 ; | 5.0 |
| *Sylvilagus audubonii* ..... | 170; | 75 ; | 14.1 ; | 18.7 |

Seasonal distribution. As shown in table 1, the flea is present on *S. audubonii* throughout the year but has summer maxima. In the first year, the average number of fleas per animal examined (8.7) was exceeded in the months of April, July, and August. In the second year, the average number (25.9) was exceeded in February, March, April, June, and July. Gravid females were most frequent in April; they were not found from November through February.

Sex ratio. During a two-year period, 58 per cent of 2,349 fleas from *S. audubonii* were females. Females exceeded males in all months except July and August of 1953 (Table 1). Reasons for a heavier infestation in the second year are not known; the increased infestation was accompanied by an increased proportion of male fleas.

2. *Hoplopsyllus affinis* (Baker)

Local distribution. Recorded only from Santa Fe Airport and other areas in the Upper Sonoran Zone.

Host records:

| | | | | |
|---|---|---|---|---|
| *Lepus californicus* ..... | 4; | 50 ; | 1.8 ; | 3.5 |
| *Sylvilagus audubonii* ..... | 170; | 79 ; | 18.7 ; | 23.5 |
| *Perognathus flavus* ..... | 989; | 1—; | 0.1—; | 1.0 |
| *Dipodomys spectabilis* ..... | 1,128; | 1—; | 0.1—; | 1.0 |
| *Peromyscus truei* ..... | 662; | 1—; | 0.1—; | 1.0 |

Seasonal distribution. Present on *S. audubonii* throughout the year but with larger collections in summer months (Table 1). In the first year, the average number of fleas per animal examined (19.3) was exceeded in the months of July, August, and September. In the second year, the average number (18.0) was exceeded in April, June, and July. Gravid females were found in all months except December.

Sex ratio. During a two-year period, 59 per cent of 3,121 fleas from *S. audubonii* were females. Females exceeded males in all months except September 1953 when only three fleas were collected.

TABLE 1

*Seasonal Abundance and Sex Ratio of Fleas from Sylvilagus audubonii at Santa Fe Airport*

| | | | *Cediopsylla i. inaequalis* | | | | *Hoplopsyllus affinis* | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Mo. | Year | Hosts Examined | Per cent Hosts Infested | Fleas Collected | Fleas Average per Host | Per cent Female Fleas | Per cent Hosts Infested | Fleas Collected | Fleas Average per Host | Per cent Female Fleas |
| Oct. | 51 | 7 | 43 | 12 | 1.7 | 83 | 57 | 36 | 5.1 | 56 |
| | 52 | 9 | 67 | 34 | 3.8 | 53 | 100 | 133 | 14.8 | 56 |
| Nov. | 51 | 0 | .. | .. | .. | .. | .. | .. | .. | .. |
| | 52 | 2 | 100 | 6 | 3.0 | 67 | 100 | 30 | 15.0 | 67 |
| Dec. | 51 | 1 | 100 | 1 | 1.0 | 100 | 100 | 6 | 6.0 | 100 |
| | 52 | 7 | 100 | 96 | 13.6 | 60 | 100 | 99 | 14.1 | 73 |
| Jan. | 52 | 2 | 100 | 6 | 3.0 | 50 | 100 | 30 | 15.0 | 53 |
| | 53 | 8 | 100 | 31 | 3.8 | 71 | 75 | 74 | 9.2 | 73 |
| Feb. | 52 | 4 | 50 | 14 | 3.5 | 64 | 100 | 23 | 5.8 | 74 |
| | 53 | 1 | 100 | 57 | 57.0 | 63 | 100 | 3 | 3.0 | 67 |
| Mar. | 52 | 7 | 86 | 25 | 3.5 | 68 | 43 | 5 | 0.7 | 80 |
| | 53 | 4 | 100 | 224 | 56.0 | 55 | 50 | 13 | 3.2 | 77 |
| Apr. | 52 | 12 | 83 | 131 | 10.9 | 64 | 58 | 64 | 5.3 | 59 |
| | 53 | 7 | 71 | 564 | 80.5 | 55 | 86 | 157 | 22.4 | 52 |
| May | 52 | 10 | 30 | 22 | 2.2 | 73 | 40 | 20 | 2.0 | 70 |
| | 53 | 4 | 50 | 19 | 4.7 | 68 | 100 | 20 | 5.0 | 70 |
| June | 52 | 16 | 69 | 66 | 4.1 | 59 | 81 | 105 | 6.6 | 66 |
| | 53 | 4 | 100 | 247 | 61.7 | 60 | 75 | 257 | 64.2 | 66 |
| July | 52 | 18 | 89 | 263 | 14.6 | 53 | 83 | 677 | 37.6 | 58 |
| | 53 | 2 | 100 | 63 | 31.5 | 44 | 100 | 137 | 68.5 | 53 |
| Aug. | 52 | 22 | 86 | 383 | 17.4 | 61 | 91 | 930 | 42.3 | 58 |
| | 53 | 2 | 50 | 31 | 15.5 | 45 | 50 | 30 | 15.0 | 50 |
| Sept. | 52 | 13 | 77 | 54 | 4.2 | 60 | 92 | 269 | 20.7 | 58 |
| | 53 | 3 | 0 | 0 | .. | .. | 100 | 3 | 1.0 | 33 |
| All | 1st | 112 | 74 | 977 | 8.7 | 60 | 76 | 2165 | 19.3 | 58 |
| | 2nd | 53 | 79 | 1372 | 25.9 | 57 | 87 | 956 | 18.0 | 61 |

### 3. *Hoplopsyllus anomalus* (Baker)

Local distribution. The few collections were from the Santa Fe Airport and other locations in the Upper Sonoran Zone.

Host records:

| | | | | |
|---|---|---|---|---|
| *Cynomys gunnisoni* ..... | 134; | 1—; | 0.1—; | 1.0 |
| *Citellus spilosoma* ..... | 326; | 1—; | 0.1—; | 1.0 |
| *Citellus variegatus* ..... | 9; | 78 ; | 5.3 ; | 6.9 |
| *Peromyscus boylei* ..... | 27; | 4 ; | 0.1—; | 1.0 |
| *Neotoma albigula* ..... | 981; | 1—; | 0.1—; | 1.0 |
| *Spilogale* sp. ..... | 2; | 50 ; | 0.5 ; | 1.0 |

Seasonal distribution. Collections were made from May through September; gravid females were observed in each month.

Sex ratio. Only 52 fleas were collected from all hosts; 54 per cent were females which exceeded males in May, July, and August. In June, 8 of 16 fleas were males and in September, 3 of 4 were males.

Holdenried and Morlan (in press) reported a pool of 15 fleas, collected from *C. variegatus* taken near Lamy on May 27, 1953, was infected with *Pasturella pestis*.

4. *Echidnophaga gallinacea* (Westwood)*

Local distribution. Recorded only from Santa Fe Airport and other Upper Sonora Zone localities.

Host records:

| | | | | |
|---|---|---|---|---|
| *Cynomys gunnisoni* ..... | 134; | 2 ; | 0.1—; | 1.7 |
| *Citellus spilosoma* ..... | 326; | 1—; | 0.1—; | 1.0 |
| *Citellus variegatus* ..... | 9; | 44 ; | 6.6 ; | 14.8 |
| *Peromyscus leucopus* ..... | 285; | 1—; | 0.1—; | 1.0 |
| *Neotoma albigula* ..... | 981; | 1—; | 0.1—; | 2.0 |
| *Mephitis* sp. ..... | 1; | 50 ; | 2.0 ; | 2.0 |

Seasonal distribution. Collected only during June through October. Gravid females were found in each of these months except September.

Sex ratio. Of 82 fleas, 87 per cent were females which exceeded males in every month.

5. *Atyphloceras echis* Jordan and Rothschild

Local distribution. Absent from extensive Santa Fe Airport collections; recorded only from Upper Sonoran Zone.

Host records:

| | | | | |
|---|---|---|---|---|
| *Peromyscus boylei* ..... | 27; | 4 ; | 0.1—; | 1.0 |
| *Peromyscus truei* ..... | 662; | 1—; | 0.1—; | 1.3 |
| *Peromyscus nasutus* ..... | 21; | 5 ; | 0.1—; | 1.0 |
| *Neotoma albigula* ..... | 981; | 1—; | 0.1—; | 1.3 |

Nest records. Of 10 *N. micropus* nests, one collected in November contained 15 male and 23 female fleas; a March collection had only 2 female fleas. *P. nasutus* did not occur at the Santa Fe Airport and no nests of this mouse were collected.

Seasonal distribution. Collected only during January, March, April, and November. A single gravid female was recorded in January.

Sex ratio. Of 18 fleas from rodents, 61 per cent were females; a single male was collected in April; females exceeded males in the other months.

6. *Hystrichopsylla dippiei* Rothschild

Local distribution. Collected only in Transition and Canadian Zones.

Host records:

| | | | | |
|---|---|---|---|---|
| *Eutamias quadrivittatus* .... | 88; | 2 ; | 0.1—; | 2.0 |
| *Neotoma cinerea* ..... | 2; | 50 ; | 0.5 ; | 1.0 |
| *Microtus longicaudus* ..... | 41; | 5 ; | 0.1—; | 1.0 |

Seasonal distribution. Collected in April, August, and November.

Sex ratio. Of 6 fleas, 67 per cent were females; the two males were collected in November and August.

7. *Catallagia decipiens* Rothschild

Local distribution. Absent from extensive Santa Fe Airport collections; recorded from Upper Sonoran and Canadian Zones.

Host records:

| | | | | |
|---|---|---|---|---|
| *Peromyscus maniculatus* ... | 445; | 1—; | 0.1—; | 2.0 |
| *Neotoma cinerea* ..... | 2; | 50 ; | 1.5 ; | 3.0 |
| *Microtus longicaudus* ..... | 41; | 2 ; | 0.1 ; | 1.0 |

Nest records. A single *M. longicaudus* nest, examined in September, 1953, contained three males and one female of this flea species.

Seasonal distribution. Collected in January and August on hosts; in September from a nest, which had a single gravid female.

Sex ratio. Of 8 fleas from rodents, 63 per cent were females; one male was collected in January and two in August.

8. *Delotelis telegoni* (Rothschild)

Only 2 male fleas were collected in January from the Canadian Zone.

Host records:

| | | | | |
|---|---|---|---|---|
| *Microtus longicaudus* ..... | 41; | 5 ; | 0.1—; | 1.0 |

9. *Epitedia stanfordi* Traub

Local distribution. Santa Fe Airport and other localities in the Upper Sonoran Zone.

Host records:

| | | | | |
|---|---|---|---|---|
| *Perognathus flavus* ..... | 989; | 1—; | 0.1—; | 1.0 |
| *Peromyscus maniculatus* ... | 445; | 3 ; | 0.1—; | 1.1 |
| *Peromyscus leucopus* ..... | 285; | 4 ; | 0.1—; | 1.1 |
| *Peromyscus truei* ..... | 662; | 6 ; | 0.1—; | 1.5 |
| *Peromyscus nasutus* ..... | 21; | 5 ; | 0.1—; | 1.0 |
| *Onychomys leucogaster* .... | 405; | 1—; | 0.1—; | 1.0 |
| *Neotoma micropus* ..... | 579; | 1—; | 0.1—; | 1.0 |
| *Neotoma albigula* ..... | 981; | 1—; | 0.1—; | 1.0 |

Nest records. Two females were taken from a *N. albigula* nest collected in October, 1951; one male and one female from a *P. leucopus* nest in March, 1953; one male and one female from a *N. micropus* nest in November, 1953.

Seasonal distribution. November through April with gravid females in each month.

Sex ratio. On rodents, females exceed males in every month; of 97 fleas, 66 per cent were females.

10. *Meringis jamesoni* Hubbard

Local distribution. Collected only at or near the Santa Fe Airport in Upper Sonoran Zone.

Host records:

| | | | | |
|---|---|---|---|---|
| *Citellus spilosoma* ..... | 326; | 1—; | 0.1—; | 1.0 |
| *Perognathus flavus* ..... | 989; | 14 ; | 0.2 ; | 1.3 |
| *Dipodomys spectabilis* ..... | 1,128; | 1—; | 0.1—; | 1.0 |
| *Peromyscus truei* ..... | 662; | 1—; | 0.1—; | 1.0 |
| *Onychomys leucogaster* .... | 405; | 4 ; | 0.1—; | 1.2 |
| *Neotoma albigula* ..... | 981; | 1—; | 0.1—; | 1.0 |

Nest records. One male and one female were taken in November from a *P. flavus* nest. The species was absent from eight other nests of *P. flavus*.

Seasonal distribution. The flea was found in only small numbers throughout the year with slight differences in seasonal abundance showing a faint tendency toward heavier infestation of *P. flavus* from June through November (Table 2). Gravid females were found in all months except January, May, and December.

TABLE 2

*Seasonal Abundance and Sex Ratio of Meringis jamesoni from Perognathus flavus at Santa Fe Airport*

| Month | Year | Hosts Examined | Per cent Hosts Infested | Fleas Collected | Fleas Average per Host* | Per cent Female Fleas |
|---|---|---|---|---|---|---|
| Oct. | 51 | 60 | 27 | 22 | 0.4 | 55 |
| | 52 | 55 | 20 | 14 | 0.3 | 64 |
| Nov. | 51 | 52 | 19 | 16 | 0.3 | 75 |
| | 52 | 28 | 14 | 4 | 0.1 | 25 |
| Dec. | 51 | 41 | 17 | 9 | 0.2 | 67 |
| | 52 | 16 | 6 | 1 | 0.1 | 0 |
| Jan. | 52 | 55 | 7 | 4 | 0.1 | 100 |
| | 53 | 25 | 0 | 0 | 0 | .. |
| Feb. | 52 | 46 | 11 | 5 | 0.1 | 80 |
| | 53 | 25 | 8 | 4 | 0.2 | 75 |
| Mar. | 52 | 63 | 6 | 4 | 0.1 | 50 |
| | 53 | 25 | 12 | 3 | 0.1 | 100 |
| Apr. | 52 | 54 | 19 | 12 | 0.2 | 67 |
| | 53 | 15 | 13 | 2 | 0.1 | 0 |
| May | 52 | 60 | 2 | 1 | 0.1– | 100 |
| | 53 | 15 | 7 | 1 | 0.1 | 100 |
| June | 52 | 75 | 7 | 6 | 0.1 | 67 |
| | 53 | 15 | 40 | 13 | 0.9 | 69 |
| July | 52 | 47 | 17 | 15 | 0.3 | 47 |
| | 53 | 15 | 27 | 5 | 0.3 | 60 |
| Aug. | 52 | 48 | 19 | 14 | 0.3 | 86 |
| | 53 | 15 | 13 | 2 | 0.1 | 0 |
| Sept. | 52 | 70 | 20 | 18 | 0.3 | 72 |
| | 53 | 15 | 20 | 3 | 0.2 | 33 |
| All | 1st | 671 | 14 | 126 | 0.2 | 67 |
| | 2nd | 264 | 15 | 52 | 0.2 | 58 |

* Averages of less than 0.05 are shown as 0.1–.

Sex ratio. During a two-year period, females comprised 65 per cent of 115 fleas from *P. flavus*. Excluding months when four or less fleas were collected, females exceeded males in all months except July, 1952 (Table 2).

11. *Meringis nidi* Williams and Hoff

Local distribution. Collections mostly from Santa Fe Airport with a few records from nearby localities in the Upper Sonoran Zone.

Host records:

| | | | | |
|---|---|---|---|---|
| *Sylvilagus audubonii* ..... | 170; | 1 ; | 0.1—; | 2.5 |
| *Citellus spilosoma* ..... | 326; | 1—; | 0.1—; | 1.0 |
| *Perognathus flavus* ..... | 989; | 1—; | 0.1—; | 1.0 |
| *Dipodomys spectabilis* ..... | 1,128; | 41 ; | 2.8 ; | 6.9 |
| *Dipodomys ordii* ..... | 2,232; | 12 ; | 0.2 ; | 1.4 |
| *Reithrodontomys megalotis* .. | 130; | 2 ; | 0.1—; | 1.0 |
| *Peromyscus maniculatus* ... | 445; | 1—; | 0.1—; | 1.0 |
| *Peromyscus truei* ..... | 662; | 1—; | 0.1—; | 1.0 |
| *Onychomys leucogaster* .... | 405; | 9 ; | 0.3 ; | 3.2 |
| *Neotoma micropus* ..... | 579; | 2 ; | 0.1—; | 1.4 |
| *Neotoma albigula* ..... | 981; | 1—; | 0.1—; | 1.1 |

Seasonal distribution. The flea reached maximum abundance in winter; it is absent from normal hosts in summer. On *D. spectabilis* (Table 4), abun-

TABLE 3

*Seasonal Abundance and Sex Ratio of Fleas from Dipodomys ordii at Santa Fe Airport*

| | | | *Meringis parkeri* | | | | *Meringis nidi* | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Mo. | Year | Hosts Examined | Per cent Hosts Infested | Fleas Collected | Fleas Average per Host* | Per cent Female Fleas | Per cent Hosts Infested | Fleas Collected | Fleas Average per Host* | Per cent Female Fleas |
| Oct. | 51 | 162 | 20 | 61 | 0.4 | 69 | 1 | 1 | 0.1– | 0 |
| | 52 | 45 | 13 | 12 | 0.3 | 75 | 4 | 2 | 0.1– | 0 |
| Nov. | 51 | 156 | 12 | 24 | 0.2 | 67 | 17 | 50 | 0.3 | 56 |
| | 52 | 25 | 12 | 6 | 0.2 | 50 | 48 | 23 | 0.9 | 52 |
| Dec. | 51 | 111 | 5 | 7 | 0.1 | 71 | 18 | 25 | 0.2 | 52 |
| | 52 | 25 | 0 | 0 | 0 | .. | 24 | 8 | 0.3 | 38 |
| Jan. | 52 | 171 | 4 | 8 | 0.1– | 63 | 23 | 64 | 0.4 | 48 |
| | 53 | 25 | 4 | 1 | 0.1– | 100 | 8 | 2 | 0.1 | 0 |
| Feb. | 52 | 180 | 5 | 11 | 0.1 | 36 | 22 | 52 | 0.3 | 33 |
| | 53 | 25 | 4 | 1 | 0.1– | 100 | 16 | 6 | 0.2 | 33 |
| Mar. | 52 | 279 | 9 | 29 | 0.1 | 48 | 19 | 65 | 0.2 | 49 |
| | 53 | 25 | 16 | 4 | 0.2 | 50 | 32 | 11 | 0.4 | 27 |
| Apr. | 52 | 149 | 13 | 27 | 0.2 | 70 | 17 | 26 | 0.2 | 35 |
| | 53 | 25 | 0 | 0 | 0 | .. | 28 | 11 | 0.4 | 45 |
| May | 52 | 169 | 9 | 20 | 0.1 | 40 | 5 | 8 | 0.1– | 13 |
| | 53 | 15 | 0 | 0 | 0 | .. | 7 | 1 | 0.1 | 100 |
| June | 52 | 133 | 4 | 5 | 0.1– | 40 | 0 | 0 | 0 | .. |
| | 53 | 15 | 0 | 0 | 0 | .. | 0 | 0 | 0 | .. |
| July | 52 | 133 | 5 | 8 | 0.1 | 25 | 0 | 0 | 0 | .. |
| | 53 | 15 | 0 | 0 | 0 | .. | 0 | 0 | 0 | .. |
| Aug. | 52 | 118 | 7 | 10 | 0.1 | 70 | 0 | 0 | 0 | .. |
| | 53 | 15 | 0 | 0 | 0 | .. | 0 | 0 | 0 | .. |
| Sept. | 52 | 95 | 11 | 17 | 0.2 | 53 | 0 | 0 | 0 | .. |
| | 53 | 15 | 0 | 0 | 0 | .. | 0 | 0 | 0 | .. |
| All | 1st | 1856 | 9 | 227 | 0.1 | 59 | 11 | 291 | 0.2 | 45 |
| | 2nd | 270 | 6 | 24 | 0.1 | 67 | 16 | 64 | 0.2 | 41 |

* Averages of less than 0.05 are shown as 0.1–.

dance exceeded the yearly averages from November through April with maxima in January and February; absent from June through September. A very similar seasonal trend was shown (Table 3) on *D. ordii*, the secondary

TABLE 4

*Seasonal Abundance and Sex Ratio of Fleas from Dipodomys spectabilis at Santa Fe Airport*

| Mo. | Year | Hosts Examined | *Meringis nidi* Per cent Hosts Infested | *Meringis nidi* Fleas Collected | *Meringis nidi* Fleas Average per Host* | *Meringis nidi* Per cent Female Fleas | *Meringis rectus* Per cent Hosts Infested | *Meringis rectus* Fleas Collected | *Meringis rectus* Fleas Average per Host* | *Meringis rectus* Per cent Female Fleas |
|---|---|---|---|---|---|---|---|---|---|---|
| Oct. | 51 | 134 | 0 | 0 | 0 | .. | 94 | 1501 | 11.2 | 48 |
| | 52 | 47 | 38 | 70 | 1.5 | 49 | 98 | 653 | 13.9 | 57 |
| Nov. | 51 | 84 | 46 | 260 | 3.1 | 55 | 77 | 275 | 3.3 | 52 |
| | 52 | 26 | 88 | 184 | 7.1 | 54 | 81 | 143 | 5.5 | 55 |
| Dec. | 51 | 52 | 89 | 219 | 4.2 | 63 | 54 | 55 | 1.1 | 51 |
| | 52 | 25 | 76 | 131 | 5.2 | 54 | 16 | 6 | 0.2 | 50 |
| Jan. | 52 | 68 | 91 | 460 | 6.8 | 52 | 29 | 46 | 0.7 | 43 |
| | 53 | 25 | 88 | 176 | 7.0 | 61 | 8 | 2 | 0.1 | 50 |
| Feb. | 52 | 59 | 88 | 375 | 6.4 | 55 | 39 | 55 | 0.9 | 44 |
| | 53 | 25 | 96 | 324 | 13.0 | 48 | 4 | 1 | 0.1– | 0 |
| Mar. | 52 | 77 | 79 | 466 | 6.1 | 58 | 81 | 316 | 4.1 | 52 |
| | 53 | 25 | 76 | 131 | 5.2 | 50 | 36 | 17 | 0.7 | 24 |
| Apr. | 52 | 48 | 83 | 178 | 3.7 | 63 | 96 | 518 | 10.8 | 43 |
| | 53 | 15 | 73 | 93 | 6.2 | 63 | 87 | 62 | 4.1 | 40 |
| May | 52 | 46 | 24 | 36 | 0.8 | 69 | 96 | 503 | 10.9 | 57 |
| | 53 | 15 | 27 | 6 | 0.4 | 83 | 100 | 31 | 2.1 | 35 |
| June | 52 | 40 | 0 | 0 | 0 | .. | 98 | 310 | 7.8 | 53 |
| | 53 | 15 | 0 | 0 | 0 | .. | 87 | 75 | 5.0 | 48 |
| July | 52 | 35 | 0 | 0 | 0 | .. | 89 | 179 | 5.1 | 53 |
| | 53 | 15 | 0 | 0 | 0 | .. | 93 | 87 | 5.8 | 45 |
| Aug. | 52 | 60 | 0 | 0 | 0 | .. | 75 | 139 | 2.3 | 58 |
| | 53 | 15 | 0 | 0 | 0 | .. | 73 | 39 | 2.6 | 56 |
| Sept. | 52 | 55 | 0 | 0 | 0 | .. | 93 | 390 | 7.1 | 48 |
| | 53 | 15 | 0 | 0 | 0 | .. | 93 | 97 | 6.5 | 67 |
| All | 1st | 758 | 41 | 1994 | 2.6 | 57 | 77 | 4287 | 5.7 | 50 |
| | 2nd | 263 | 53 | 1115 | 4.2 | 54 | 62 | 1213 | 4.6 | 54 |

* Averages of less than 0.05 are shown as 0.1–.

host. Gravid females were found in all months that the flea was collected; over 80 per cent of the females examined were gravid from February through May of each year.

Sex ratio (Tables 3 and 4). Of 3,109 fleas collected from *D. spectabilis* during a two-year period, 56 per cent were females; females exceeded males in all months except October, 1952, and February, 1953. Females accounted for 44 per cent of the 355 fleas collected from *D. ordii* during a two-year study at the Santa Fe Airport; in months when several fleas were collected, males predominated except in November of both years and December of 1951.

12. *Meringis parkeri* Jordan

Local distribution. Upper Sonoran Zone only, including Santa Fe Airport.

Host records:

| | | | | | Per cent Female Fleas |
|---|---|---|---|---|---|
| *Citellus spilosoma* ..... | 326; | 1—; | 0.1—; | 1.0 | 48 |
| *Perognathus flavus* ..... | 989; | 1—; | 0.1—; | 2.5 | 57 |
| *Dipodomys spectabilis* ..... | 1,128; | 1—; | 0.1—; | 1.0 | 52 |
| *Dipodomys ordii* ..... | 2,232; | 9 ; | 0.1 ; | 1.4 | 55 |
| *Peromyscus maniculatus* ... | 445; | 1—; | 0.1—; | 1.0 | 51 |
| *Onychomys leucogaster* .... | 405; | 9 ; | 0.2 ; | 2.1 | 50 |
| *Neotoma micropus* ..... | 579; | 1 ; | 0.1—; | 1.0 | 43 |
| *Neotoma albigula* ..... | 981; | 1—; | 0.1—; | 1.0 | 50 |

Although *D. ordii* and *O. leucogaster* show similar average infestations, the primary host may prove to be the former as indicated below by nest and sex ratio records.

Nest records. Three male and three female fleas were collected in February from a *D. ordii* nest.

Seasonal distribution. This flea seems to reach a maximum in late summer and fall. It is found in small numbers on *D. ordii* during all months of the year with maximum abundance in October (Table 3). On *O. leucogaster* (Table 5), heavier infestation was observed in August through November; the flea was absent from this host from January through March. Small numbers of females were examined in all months except December; gravid females were found in all months except March, June, and July.

Sex ratio. During a two-year period, females accounted for 60 per cent of 251 fleas from *D. ordii* but only 40 per cent of 73 fleas from *O. leucogaster*. This may indicate that *D. ordii* is the primary host. On *D. ordii*, females were predominant in the small collections from August through January.

13. *Meringis rectus* Morlan

Local distribution. Upper Sonoran Zone only, including Santa Fe Airport.

Host records:

| | | | | | |
|---|---|---|---|---|---|
| *Sylvilagus audubonii* ..... | 170; | 1 ; | 0.1—; | 2.0 | 48 |
| *Cynomys gunnisoni* ..... | 134; | 2 ; | 0.1—; | 1.0 | 67 |
| *Citellus spilosoma* ..... | 326; | 1 ; | 0.1—; | 1.5 | 50 |
| *Citellus variegatus* ..... | 9; | 11 ; | 0.1 ; | 1.1 | 54 |
| *Perognatus flavus* ..... | 989; | 1—; | 0.1—; | 1.1 | |
| *Dipodomys spectabilis* ..... | 1,128; | 65 ; | 5.5 ; | 7.9 | |
| *Dipodomys ordii* ..... | 2,232; | 3 ; | 0.1—; | 1.4 | |
| *Reithrodontomys megalotis*.. | 130; | 1—; | 0.1—; | 1.0 | |
| *Peromyscus maniculatus*.... | 445; | 1—; | 0.1—; | 1.0 | |
| *Peromyscus truei* ..... | 662; | 1—; | 0.1—; | 1.0 | |
| *Onychomys leucogaster* .... | 405; | 9 ; | 0.1 ; | 1.4 | |
| *Neotoma micropus* ..... | 579; | 1—; | 0.1—; | 1.0 | |
| *Neotoma albigula* ..... | 981; | 1 ; | 0.1—; | 1.0 | |

Nest records. A total of seven male and two female fleas were collected from four *D. spectabilis* nests taken in January, February, May, and November.

TABLE 5

*Seasonal Abundance and Sex Ratio of Fleas from Onychomys leucogaster at Santa Fe Airport*

| | | | | *Meringis parkeri* | | | | *Monopsyllus exilis* | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Mo. | Year | Hosts Examined | Per cent Hosts Infested | Fleas Collected | Fleas Average per Host | Per cent Female Fleas | Per cent Hosts Infested | Fleas Collected | Fleas Average per Host | Per cent Female Fleas |
| Oct. | 51 | 16 | 13 | 6 | 0.4 | 0 | 44 | 23 | 1.4 | 57 |
| | 52 | 30 | 13 | 13 | 0.4 | 54 | 20 | 11 | 0.4 | 55 |
| Nov. | 51 | 7 | 29 | 6 | 0.9 | 33 | 57 | 10 | 1.4 | 70 |
| | 52 | 25 | 0 | 0 | 0 | .. | 16 | 8 | 0.3 | 63 |
| Dec. | 51 | 1 | 0 | 0 | 0 | .. | 100 | 1 | 1.0 | 100 |
| | 52 | 21 | 10 | 2 | 0.1 | 50 | 19 | 4 | 0.2 | 100 |
| Jan. | 52 | 1 | 0 | 0 | 0 | .. | 0 | 0 | 0 | .. |
| | 53 | 14 | 0 | 0 | 0 | .. | 0 | 0 | 0 | .. |
| Feb. | 52 | 3 | 0 | 0 | 0 | .. | 0 | 0 | 0 | .. |
| | 53 | 16 | 0 | 0 | 0 | .. | 0 | 0 | 0 | .. |
| Mar. | 52 | 7 | 0 | 0 | 0 | .. | 14 | 1 | 0.1 | 0 |
| | 53 | 25 | 0 | 0 | 0 | .. | 8 | 2 | 0.1 | 0 |
| Apr. | 52 | 13 | 8 | 1 | 0.1 | 0 | 31 | 4 | 0.3 | 75 |
| | 53 | 15 | 0 | 0 | 0 | .. | 27 | 5 | 0.3 | 20 |
| May | 52 | 14 | 14 | 2 | 0.1 | 50 | 0 | 0 | 0 | .. |
| | 53 | 12 | 0 | 0 | 0 | .. | 33 | 5 | 0.4 | 80 |
| June | 52 | 22 | 5 | 2 | 0.1 | 50 | 55 | 32 | 1.5 | 56 |
| | 53 | 15 | 0 | 0 | 0 | .. | 67 | 51 | 3.4 | 55 |
| July | 52 | 18 | 11 | 3 | 0.2 | 33 | 50 | 55 | 3.0 | 47 |
| | 53 | 15 | 0 | 0 | 0 | .. | 47 | 28 | 1.8 | 50 |
| Aug. | 52 | 21 | 19 | 6 | 0.3 | 67 | 38 | 18 | 0.8 | 44 |
| | 53 | 15 | 7 | 3 | 0.2 | 33 | 60 | 45 | 3.0 | 64 |
| Sept. | 52 | 46 | 17 | 22 | 0.5 | 36 | 30 | 27 | 0.6 | 44 |
| | 53 | 16 | 19 | 7 | 0.4 | 43 | 69 | 24 | 1.5 | 33 |
| All | 1st | 169 | 13 | 48 | 0.3 | 35 | 35 | 171 | 1.0 | 51 |
| | 2nd | 219 | 5 | 25 | 0.1 | 48 | 28 | 183 | 0.8 | 54 |

Seasonal distribution. Peaks of abundance occurred in spring or early summer and fall. Maximum abundance was observed on *D. spectabilis* (Table 4) in October and a marked seasonal decline was noted from December through March and again in August. The observed trend is in notable contrast to that of *M. nidi* which has the same primary host but increases and declines at nearly opposite seasons. Gravid females were observed throughout the year with a reduction in proportion of gravid females apparent from December through February.

Sex ratio. Of 5,500 fleas from *D. spectabilis* in a two-year period, 51 per cent were females. There was no apparent explanation for the irregular monthly variations in sex ratio (Table 4).

14. *Phalacropsylla* sp.

Host records:

*Ochotona princeps* ..... 9; 11 ; 0.1 ; 1.0

A single male, possibly a new species, was taken in the Canadian Zone during October, 1953.

15. *Rhadinopsylla sectilis sectilis* (Jordan and Rothschild)

| | | | | |
|---|---|---|---|---|
| *Peromyscus maniculatus*.... | 445; | 1—; | 0.1—; | 2.0 |
| *Peromyscus nasutus* ..... | 21; | 5 ; | 0.1—; | 1.0 |

A single female was collected from *P. nasutus* in May, 1952, and two males from *P. maniculatus* in April, 1953; all were from Upper Sonoran Zone and not found in the extensive Santa Fe Airport collections.

16. *Rhadinopsylla multidenticulatus* Morlan and Prince

Local distribution. All collections were from or near the Santa Fe Airport in the Upper Sonoran Zone.

Host records:

| | | | | |
|---|---|---|---|---|
| *Dipodomys spectabilis* ..... | 1,128; | 1 ; | 0.1—; | 1.4 |
| *Dipodomys ordii* ..... | 2,232; | 1—; | 0.1—; | 1.2 |
| *Onychomys leucogaster* .... | 405; | 7 ; | 0.1 ; | 1.6 |
| *Neotoma micropus* ..... | 579; | 1—; | 0.1—; | 1.5 |
| *Neotoma albigula* ..... | 981; | 1—; | 0.1—; | 1.0 |

Nest records. Two nests of *N. albigula* taken in November and December contained three males and one female. Two nests of *D. spectabilis* collected in February provided seven males and 14 females. A single *D. ordii* nest had two males in February.

Seasonal distribution. The species is most prevalent from November through January; it is absent from collections of June through September. Of 17 females examined in December and January, only two, collected in December, were gravid.

Sex ratio. Of 73 fleas from all hosts, 59 per cent were females; females outnumbered males in October, November, March, and May.

17. *Rhadinopsylla fraterna* (Baker)

| | | | | |
|---|---|---|---|---|
| *Cleithrionomys gapperi* .... | 4; | 25 ; | 0.3 ; | 1.0 |

A single male was taken in October, 1953, from the Canadian Zone.

18. *Anomiopsyllus nudatus* (Baker)

Local distribution. Upper Sonoran (including Santa Fe Airport) and Transition Zones.

Host records:

| | | | | |
|---|---|---|---|---|
| *Thomomys talpoides* ..... | 15; | 7 ; | 0.1—; | 1.0 |
| *Perognathus flavus* ..... | 989; | 1—; | 0.1—; | 1.0 |
| *Peromyscus leucopus* ..... | 285; | 1—; | 0.1—; | 1.0 |
| *Peromyscus truei* ..... | 662; | 1—; | 0.1—; | 1.0 |
| *Neotoma micropus* ..... | 579; | 3 ; | 0.1—; | 1.4 |
| *Neotoma albigula* ..... | 981; | 3 ; | 0.1—; | 2.6 |
| *Neotoma mexicana* ..... | 9; | 22 ; | 0.8 ; | 3.5 |
| *Spilogale* sp. ..... | 2; | 50 ; | 2.0 ; | 4.0 |

Nest records. Of 28 *N. albigula* nests examined, 26 were infested with 580 fleas; 60 per cent of which were females. Collections were made during every

month of the year, over 100 fleas were recovered from a single nest in November and in January but only light infestations were found from March through June. This flea was also found in seven of 10 *N. micropus* nests. Two of five *P. truei* nests were infested, probably by location in a former *N. albigula* habitat. The single nest of *P. leucopus* that was examined contained a single female of *A. nudatus*.

Flea larvae were frequently present in rodent nests but occasional attempts to rear adults in the laboratory were only partially successful. About 500 larvae, collected April 23, 1952, from a *N. micropus* nest, produced *A. nudatus* adults observed as follows: May 24, one male; June 12, 19 males and 26 females; June 16, one male and four females. On November 18, 1952, 94 larvae were collected from a *N. albigula* nest; a single adult female was found on December 10. On March 24, 1953, over 100 larvae were obtained from a *N. albigula* nest; two males were observed on May 8, 1953.

19. *Callistopsyllus terinus* (Rothschild)

| | | | | |
|---|---|---|---|---|
| *Peromyscus maniculatus*.... | 445; | 1—; | 0.1—; | 6.0 |
| *Peromyscus truei* ..... | 662; | 1—; | 0.1—; | 2.0 |

Only 8 specimens have been collected, all in the Upper Sonoran Zone near Pojoaque during April, 1953. One male and one female were from *P. truei*; four males and two females from *P. maniculatus*.

20. *Megarthroglossus bisetis* Jordan and Rothschild

Host records:

| | | | | |
|---|---|---|---|---|
| *Thomomys talpoides* ..... | 15; | 7 ; | 0.1—; | 1.0 |
| *Peromyscus truei* ..... | 662; | 1—; | 0.1—; | 1.0 |
| *Peromyscus nasutus* ..... | 21; | 5 ; | 0.1—; | 1.0 |
| *Neotoma albigula* ..... | 981; | 1—; | 0.1—; | 2.5 |

Only 10 specimens were collected from Upper Sonoran and Transition Zones: one female in August, three males and two females in September, three males and one female in January. The species was absent from extensive Santa Fe Airport collections. One of the two females collected in September was gravid; the single female collected in January was not gravid.

Nest records. Six of 10 *N. micropus* nests had a total of 107 male and 110 female fleas. In November, 1953, 5 of 6 *N. micropus* nests contained this "rare" species; one nest had 84 male and 95 female fleas. Six of 28 *N. albigula* nests had a total of seven males and nine females. All nest collections were in January, March, October, and November.

21. *Megarthroglossus divisus divisus* (Baker)

Host records:

| | | | | |
|---|---|---|---|---|
| *Ochotona princeps* ..... | 9; | 22 ; | 0.2 ; | 1.0 |
| *Tamiasciurus hudsonicus* .. | 9; | 11 ; | 0.1 ; | 1.0 |
| *Peromyscus maniculatus* .... | 445; | 1—; | 0.1—; | 1.0 |
| *Peromyscus leucopus* ..... | 285; | 1—; | 0.1—; | 1.0 |
| *Neotoma albigula* ..... | 981; | 1—; | 0.1—; | 2.0 |

Seven specimens were collected from Upper Sonoran (including Airport),

Transition, and Canadian Zones in February, May, August, September, and October. The single female was taken in October.

Nest records. A male and a female were taken from a *N. albigula* nest collected in January; a single male from a *N. micropus* nest in March; a single female, from a *T. hudsonicus* nest in October, was gravid.

22. *Stenistomera alpina* (Baker)

Local distribution. Upper Sonoran and Transition Zones. Absent from extensive Santa Fe Airport collections.

Host records:

| | | | | |
|---|---|---|---|---|
| *Eutamias quadrivittatus* .... | 88; | 1 ; | 0.1—; | 1.0 |
| *Neotoma albigula* ..... | 981; | 1 ; | 0.1 ; | 8.4 |
| *Neotoma mexicana* ..... | 9; | 33 ; | 2.9 ; | 8.7 |
| *Neotoma cinerea* ..... | 2; | 50 ; | 1.0 ; | 2.0 |

Nest records. A single male was found in each of two *N. albigula* nests collected in December and January.

Seasonal distribution. Collected in September, November, January, February, March, and April and gravid females were observed in each of these months. A single woodrat (*N. albigula*), collected February 4, 1954, in the San Pedro Mountains south of Santa Fe, yielded 82 fleas of this species.

Sex ratio. Of 136 fleas, 56 per cent were females.

23. *Stenistomera macrodactyla* Good

| | | | | |
|---|---|---|---|---|
| *Neotoma albigula* ..... | 981; | 1—; | 0.1—; | 1.0 |

A single specimen, collected from the Upper Sonoran Zone (near Lamy) in June, was infected with *P. pestis* (Holdenried and Morlan, in press).

24. *Ctenophyllus terribilis* (Rothschild)

| | | | | |
|---|---|---|---|---|
| *Ochotona princeps* ..... | 9; | 33 ; | 1.3 ; | 4.0 |
| *Thomomys talpoides* ..... | 15; | 7 ; | 0.1—; | 1.0 |

Of 13 fleas collected in August and October in the Canadian Zone, 69 per cent were females. One of the five females examined in October was gravid.

25. *Odontopsyllus dentatus* (Baker)

| | | | | |
|---|---|---|---|---|
| *Sylvilagus audubonii* ..... | 170; | 2 ; | 0.1—; | 1.7 |

Only one male and four females were collected during March and April from the Santa Fe Airport in the Upper Sonoran Zone. In April, two of the three females examined were gravid.

26. *Amphalius necopinus* (Jordan)

| | | | | |
|---|---|---|---|---|
| *Ochotona princeps* ..... | 9; | 22 ; | 0.2 ; | 3.0 |

Four male and two female fleas were collected in October from the Canadian Zone; the females were not gravid.

27. *Diamanus montanus* (Baker)*

Local distribution. Upper Sonoran and Transition Zones; absent from Santa Fe Airport.

Host records:

| | | | | |
|---|---|---|---|---|
| *Citellus variegatus* ..... | 9; | 67 ; | 4.8 ; | 7.2 |
| *Citellus lateralis* ..... | 29; | 10 ; | 0.1 ; | 3.0 |
| *Reithrodontomys megalotis*.. | 130; | 1—; | 0.1—; | 1.0 |
| *Peromyscus truei* ..... | 662; | 1—; | 0.1—; | 1.0 |
| *Neotoma albigula* ..... | 981; | 1—; | 0.1—; | 1.0 |
| *Spilogale* sp. ..... | 2; | 50 ; | 0.5 ; | 1.0 |

Nest records. A single male of this flea was removed from a *N. albigula* nest collected in November, 1951.

Seasonal distribution. April, May, July, August, September, and November. Gravid females were found in each month except July.

Sex ratio. Of 49 fleas, 53 per cent were females.

### 28. *Foxella ignota* (Baker)

Local distribution. Upper Sonoran, Transition, and Canadian Zones; absent from Santa Fe Airport collections.

Host records:

| | | | | |
|---|---|---|---|---|
| *Thomomys talpoides* ..... | 15; | 93 ; | 5.1 ; | 5.5 |
| *Thomomys bottae* ..... | 6; | 67 ; | 6.3 ; | 9.5 |

Seasonal distribution. March and August through November. Gravid females were noted in March, October, and November.

Sex ratio. Of 115 fleas, 58 per cent were females.

### 29. *Malaraeus euphorbi* (Rothschild)

Host records:

| | | | | |
|---|---|---|---|---|
| *Peromyscus maniculatus* ... | 445; | 1—; | 0.1—; | 1.0 |
| *Peromyscus truei* ..... | 662; | 1—; | 0.1—; | 1.5 |
| *Cleithrionomys gapperi* .... | 4; | 25 ; | 0.3 ; | 1.0 |

Only 9 fleas taken during October, December, and January in both Upper Sonoran (including Santa Fe Airport) and Canadian Zones; 78 per cent were females.

### 30. *Malaraeus* sp. n.

| | | | | |
|---|---|---|---|---|
| *Neotoma micropus* ..... | 579; | 1—; | 0.1—; | 1.0 |

A single male was collected in January from La Bajada (about 19 miles southwest of Santa Fe) in the Upper Sonoran Zone.

### 31. *Malaraeus sinomus* (Jordan)

Local distribution. Upper Sonoran Zone; absent from extensive Santa Fe Airport collections.

Host records:

| | | | | |
|---|---|---|---|---|
| *Peromyscus maniculatus*.... | 445; | 1—; | 0.1—; | 1.0 |
| *Peromyscus leucopus* ..... | 285; | 1—; | 0.1—; | 1.0 |
| *Peromyscus boylei* ..... | 27; | 15 ; | 0.2 ; | 1.5 |
| *Peromyscus truei* ..... | 662; | 2 ; | 0.1—; | 1.1 |
| *Peromyscus nasutus* ..... | 21; | 5 ; | 0.1—; | 1.0 |
| *Onychomys leucogaster* .... | 405; | 1—; | 0.1—; | 1.0 |

| | | | | |
|---|---|---|---|---|
| *Neotoma albigula* ..... | 981; | 1—; | 0.1—; | 1.0 |
| *Neotoma mexicana* ..... | 9; | 11 ; | 0.1 ; | 1.0 |

Seasonal distribution. Of 14 fleas, 12 were collected in April; one each in January and August. Four of six females collected in April were gravid.

Sex ratio. Of 14 fleas, 58 per cent were females.

32. *Malaraeus telchinum* (Rothschild)*

| | | | | |
|---|---|---|---|---|
| *Peromyscus maniculatus* ... | 445; | 1—; | 0.1—; | 1.0 |
| *Microtus longicaudus* ..... | 41; | 2 ; | 0.1—; | 1.0 |

Only two males were collected during September in the Transition Zone.

33. *Megabothris abantis* (Rothschild)*

| | | | | |
|---|---|---|---|---|
| *Ochotona princeps* ..... | 9; | 11 ; | 0.1 ; | 1.0 |
| *Microtus longicaudus* ..... | 41; | 5 ; | 0.1—; | 1.0 |

Only two males and one female collected during August and September in Transition and Canadian Zones.

Nest records. Three male and two female fleas were removed from the single *M. longicaudus* nest examined in September, 1953; both females were gravid.

34. *Monopsyllus eumolpi* (Rothschild)*

Local distribution. Upper Sonoran, Transition, and Canadian Zones; absent from Santa Fe Airport collections.

Host records:

| | | | | |
|---|---|---|---|---|
| *Tamiasciurus hudsonicus* ... | 9; | 22 ; | 0.2 ; | 1.5 |
| *Citellus lateralis* ..... | 29; | 3 ; | 0.1—; | 1.0 |
| *Eutamias minimus* ..... | 19; | 32 ; | 0.4 ; | 1.3 |
| *Eutamias quadrivittatus* .... | 88; | 32 ; | 0.6 ; | 1.9 |
| *Thomomys talpoides* ..... | 15; | 7 ; | 0.1—; | 1.0 |
| *Peromyscus maniculatus*.... | 445; | 1—; | 0.1—; | 1.0 |
| *Neotoma mexicana* ..... | 9; | 11 ; | 0.1 ; | 1.0 |
| *Microtus longicaudus* ..... | 41; | 10 ; | 0.1—; | 1.0 |

Seasonal distribution. Largest numbers were collected in August and September; a few records occurred in February, March, May, October, and November. Of 15 females examined, none were gravid.

Sex ratio. Of 73 fleas, 51 per cent were females. However, only 43 per cent of the 37 fleas collected in August were females.

35. *Monopsyllus exilis* (Jordan)

Local distribution. All collections were from the Upper Sonoran Zone, mostly from the Santa Fe Airport.

Host records:

| | | | | |
|---|---|---|---|---|
| *Lepus californicus* ..... | 4; | 25 ; | 0.3 ; | 1.0 |
| *Dipodomys spectabilis* ..... | 1,128; | 1—; | 0.1—; | 1.0 |
| *Dipodomys ordii* ..... | 2,232; | 1—; | 0.1—; | 1.0 |
| *Peromyscus leucopus* ..... | 285; | 1—; | 0.1—; | 1.0 |
| *Onychomys leucogaster* .... | 405; | 32 ; | 0.9 ; | 2.9 |
| *Neotoma micropus* ..... | 579; | 1—; | 0.1—; | 1.0 |

| | | | | |
|---|---|---|---|---|
| *Neotoma albigula* ..... | 981; | 1—; | 0.1—; | 1.0 |
| *Neotoma mexicana* ..... | 9; | 11 ; | 0.2 ; | 2.0 |

Seasonal distribution. Apparently a summer flea, it reached maximum abundance on *O. leucogaster* (Table 5), in June and July and was entirely absent from this host in January and February. Most of the females examined from April through December were gravid.

Sex ratio. Of 354 fleas from *O. leucogaster* during a two-year period, 53 per cent were females. Except for September, females predominated in all months of either the first or second year's observations (Table 5).

### 36. *Monopsyllus vison* (Baker)

| | | | | |
|---|---|---|---|---|
| *Tamiasciurus hudsonicus*.... | 9; | 11 ; | 0.1 ; | 1.0 |
| *Peromyscus nasutus*..... | 21; | 5 ; | 0.1—; | 1.0 |

Only two specimens were collected; one male from the Upper Sonoran Zone in August and one female from the Transition Zone in September.

Nest records. Of four *T. hudsonicus* nests taken in October, 1953, two were infested with a total of 18 male and 10 female fleas. Only five females were examined; none were gravid.

### 37. *Monopsyllus wagneri* (Baker)

Local distribution. Many records throughout Upper Sonoran (including Santa Fe Airport), Transition, and Canadian Zones.

Host records:

| | | | | |
|---|---|---|---|---|
| *Sylvilagus audubonii* ..... | 170; | 1—; | 0.1—; | 1.0 |
| *Citellus spilosoma*..... | 326; | 1—; | 0.1—; | 1.0 |
| *Eutamias quadrivittatus* .... | 88; | 1 ; | 0.1—; | 1.0 |
| *Dipodomys ordii* ..... | 2,232; | 1—; | 0.1—; | 1.0 |
| *Reithrodontomys megalotis*.. | 130; | 9 ; | 0.1 ; | 1.6 |
| *Peromyscus maniculatus*.... | 445; | 20 ; | 0.4 ; | 1.9 |
| *Peromyscus leucopus* ..... | 285; | 29 ; | 0.7 ; | 2.3 |
| *Peromyscus boylei* ..... | 27; | 19 ; | 0.2 ; | 1.2 |
| *Peromyscus truei* ..... | 662; | 31 ; | 0.8 ; | 2.5 |
| *Peromyscus nasutus* ..... | 21; | 10 ; | 0.2 ; | 2.0 |
| *Onychomys leucogaster* .... | 405; | 1—; | 0.1—; | 1.0 |
| *Neotoma micropus* ..... | 579; | 1—; | 0.1—; | 1.0 |
| *Neotoma albigula* ..... | 981; | 1—; | 0.1—; | 1.0 |
| *Neotoma cinera* ..... | 2; | 100 ; | 1.0 ; | 1.0 |
| *Microtus longicaudus* ..... | 41; | 2 ; | 0.1—; | 1.0 |

Nest records. A *P. truei* nest taken in May had one male and one female. Three male and two female fleas were removed from a *M. longicaudus* nest collected in September.

Seasonal distribution. Records from *P. truei* (Table 6), *P. maniculatus* (Table 7), and *P. leucopus* (Table 8) indicate a minimum abundance in June and July with a sharp decline in numbers during winter months. The flea was collected in all months of the year from *P. maniculatus;* it was absent from *P. truei* collections of September, November, and December; and was not collected from *P. leucopus* in either December or January. Gravid

TABLE 6

*Seasonal Abundance and Sex Ratio of Fleas from Peromyscus truei at Santa Fe Airport*

| | | | *Monopsyllus wagneri* | | | | *Orchopeas leucopus* | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Mo. | Year | Hosts Examined | Per cent Hosts Infested | Fleas Collected | Fleas Average per Host | Per cent Female Fleas | Per cent Hosts Infested | Fleas Collected | Fleas Average per Host | Per cent Female Fleas |
| Oct. | 51 | 13 | 38 | 17 | 1.3 | 59 | 54 | 14 | 1.1 | 57 |
| | 52 | 3 | 33 | 1 | 0.3 | 100 | 33 | 2 | 0.7 | 100 |
| Nov. | 51 | 12 | 0 | 0 | 0 | .. | 25 | 3 | 0.2 | 100 |
| | 52 | 8 | 0 | 0 | 0 | .. | 38 | 4 | 0.5 | 75 |
| Dec. | 51 | 4 | 0 | 0 | 0 | .. | 75 | 3 | 0.8 | 67 |
| | 52 | 8 | 0 | 0 | 0 | .. | 13 | 1 | 0.1 | 0 |
| Jan. | 52 | 20 | 5 | 1 | 0.1 | 100 | 15 | 4 | 0.2 | 50 |
| | 53 | 7 | 0 | 0 | 0 | .. | 14 | 1 | 0.1 | 0 |
| Feb. | 52 | 15 | 20 | 3 | 0.2 | 67 | 20 | 4 | 0.3 | 50 |
| | 53 | 9 | 0 | 0 | 0 | .. | 11 | 1 | 0.1 | 100 |
| Mar. | 52 | 18 | 17 | 3 | 0.2 | 100 | 39 | 17 | 0.9 | 65 |
| | 53 | 25 | 0 | 0 | 0 | .. | 28 | 10 | 0.4 | 50 |
| Apr. | 52 | 30 | 20 | 6 | 0.2 | 67 | 60 | 36 | 1.2 | 75 |
| | 53 | 15 | 0 | 0 | 0 | .. | 73 | 33 | 2.2 | 48 |
| May | 52 | 18 | 28 | 12 | 0.7 | 58 | 61 | 28 | 1.6 | 64 |
| | 53 | 15 | 27 | 6 | 0.4 | 67 | 93 | 62 | 4.1 | 58 |
| June | 52 | 10 | 40 | 9 | 0.9 | 67 | 40 | 9 | 0.9 | 78 |
| | 53 | 11 | 45 | 9 | 0.8 | 44 | 91 | 45 | 4.1 | 71 |
| July | 52 | 2 | 50 | 2 | 1.0 | 50 | 50 | 7 | 3.5 | 57 |
| | 53 | 15 | 47 | 15 | 1.0 | 60 | 53 | 16 | 1.1 | 75 |
| Aug. | 52 | 2 | 50 | 3 | 1.5 | 33 | 50 | 1 | 0.5 | 100 |
| | 53 | 8 | 25 | 2 | 0.2 | 100 | 63 | 13 | 1.6 | 69 |
| Sept. | 52 | 1 | 0 | 0 | 0 | .. | 0 | 0 | 0 | .. |
| | 53 | 12 | 0 | 0 | 0 | .. | 33 | 11 | 0.9 | 64 |
| All | 1st | 145 | 20 | 56 | 0.4 | 63 | 42 | 126 | 0.9 | 67 |
| | 2nd | 136 | 14 | 33 | 0.2 | 61 | 49 | 199 | 1.5 | 62 |

females were recorded from March through November; the few examined in January and February were not gravid; none were examined in December.

Sex ratio. Females accounted for about 60 per cent of the fleas collected from each of the three hosts as shown in tables 6, 7, and 8. There was no apparent pattern to the monthly variations in sex ratio. In the few months when females did not predominate, the records were either inadequate in number or were for just one of two years.

Several pools of this species, collected near Lamy in May, were infected with *P. pestis* (Holdenried and Morlan, in press).

38. *Opisocrostis hirsutus* (Baker)*

Local distribution. Upper Sonoran Zone. The principal host (*C. gunnisoni*), although present, was not trapped at the Santa Fe Airport. All records are from St. Michael's College.

TABLE 7

*Seasonal Abundance and Sex Ratio of Fleas from Peromyscus maniculatus at Santa Fe Airport*

| | | | *Monopsyllus wagneri* | | | | *Orchopeas leucopus* | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Mo. | Year | Hosts Examined | Per cent Hosts Infested | Fleas Collected | Fleas Average per Host | Per cent Female Fleas | Per cent Hosts Infested | Fleas Collected | Fleas Average per Host | Per cent Female Fleas |
| Oct. | 51 | 11 | 45 | 15 | 1.4 | 47 | 18 | 2 | 0.2 | 100 |
| | 52 | 15 | 13 | 3 | 0.2 | 67 | 7 | 1 | 0.1 | 100 |
| Nov. | 51 | 11 | 9 | 2 | 0.2 | 50 | 9 | 1 | 0.1 | 100 |
| | 52 | 10 | 20 | 2 | 0.2 | 50 | 0 | 0 | 0 | .. |
| Dec. | 51 | 7 | 29 | 2 | 0.3 | 100 | 29 | 2 | 0.3 | 50 |
| | 52 | 10 | 0 | 0 | 0 | .. | 10 | 1 | 0.1 | 0 |
| Jan. | 52 | 22 | 9 | 2 | 0.1 | 50 | 27 | 7 | 0.3 | 86 |
| | 53 | 15 | 0 | 0 | 0 | .. | 0 | 0 | 0 | .. |
| Feb. | 52 | 29 | 7 | 2 | 0.1 | 100 | 17 | 5 | 0.2 | 80 |
| | 53 | 18 | 11 | 4 | 0.2 | 50 | 11 | 3 | 0.2 | 67 |
| Mar. | 52 | 39 | 5 | 2 | 0.1 | 0 | 44 | 49 | 1.3 | 67 |
| | 53 | 23 | 13 | 4 | 0.2 | 75 | 9 | 4 | 0.2 | 75 |
| Apr. | 52 | 31 | 13 | 4 | 0.1 | 100 | 29 | 16 | 0.5 | 63 |
| | 53 | 19 | 5 | 1 | 0.1 | 100 | 26 | 5 | 0.3 | 80 |
| May | 52 | 22 | 32 | 10 | 0.5 | 60 | 18 | 7 | 0.3 | 57 |
| | 53 | 15 | 40 | 7 | 0.5 | 86 | 33 | 9 | 0.6 | 44 |
| June | 52 | 8 | 50 | 11 | 1.4 | 55 | 38 | 5 | 0.6 | 40 |
| | 53 | 11 | 55 | 13 | 1.2 | 62 | 18 | 4 | 0.4 | 50 |
| July | 52 | 3 | 33 | 1 | 0.3 | 0 | 33 | 1 | 0.3 | 0 |
| | 53 | 9 | 44 | 4 | 0.4 | 75 | 11 | 2 | 0.2 | 50 |
| Aug. | 52 | 2 | 0 | 0 | 0 | .. | 0 | 0 | 0 | .. |
| | 53 | 10 | 20 | 3 | 0.3 | 33 | 40 | 11 | 1.1 | 64 |
| Sept. | 52 | 3 | 33 | 1 | 0.3 | 100 | 0 | 0 | 0 | .. |
| | 53 | 4 | 50 | 3 | 0.8 | 67 | 50 | 2 | 0.5 | 0 |
| All | 1st | 188 | 16 | 52 | 0.3 | 58 | 27 | 95 | 0.5 | 66 |
| | 2nd | 159 | 19 | 44 | 0.3 | 66 | 16 | 42 | 0.3 | 57 |

Host records:

| | | | | |
|---|---|---|---|---|
| *Cynomys gunnisoni* ..... | 134; | 56 ; | 4.3 ; | 7.6 |
| *Citellus variegatus* ..... | 9; | 11 ; | 1.1 ; | 10.0 |

Seasonal distribution. The principal host, *C. gunnisoni*, was in hibernation from October through February. No records of this flea were obtained during this period. The flea apparently is less abundant during May and June than in the months preceding and following hibernation of the host (Table 9). Gravid females were frequently observed from March through September.

Sex ratio. Of the 461 fleas taken from *C. gunnisoni* in 1952, 57 per cent were females. Females were predominant in every month except September, 1952 (Table 9); however, in September, 1953, 54 per cent of 28 fleas from the same host and location were female.

TABLE 8

*Seasonal Abundance and Sex Ratio of Fleas from Peromyscus leucopus at Santa Fe Airport*
*October 1952–September 1953*

| Month | Hosts Examined | *Monopsyllus wagneri* Per cent Hosts Infested | Fleas Collected | Fleas Average per Host | Per cent Female Fleas | *Orchopeas leucopus* Per cent Hosts Infested | Fleas Collected | Fleas Average per Host | Per cent Female Fleas |
|---|---|---|---|---|---|---|---|---|---|
| Oct. | 10 | 0 | 0 | 0 | .. | 30 | 3 | 0.3 | 67 |
| Nov. | 13 | 15 | 3 | 0.2 | 67 | 23 | 4 | 0.3 | 50 |
| Dec. | 3 | 0 | 0 | 0 | .. | 0 | 0 | 0 | .. |
| Jan. | 13 | 0 | 0 | 0 | .. | 0 | 0 | 0 | .. |
| Feb. | 21 | 14 | 6 | 0.3 | 83 | 10 | 2 | 0.1 | 50 |
| Mar. | 24 | 0 | 0 | 0 | .. | 21 | 6 | 0.2 | 83 |
| Apr. | 18 | 6 | 1 | 0.1 | 100 | 61 | 22 | 1.2 | 91 |
| May | 15 | 27 | 8 | 0.5 | 75 | 53 | 23 | 1.5 | 83 |
| June | 15 | 73 | 24 | 1.6 | 50 | 87 | 45 | 3.0 | 67 |
| July | 16 | 69 | 30 | 1.9 | 53 | 63 | 32 | 2.0 | 59 |
| Aug. | 18 | 56 | 17 | 0.9 | 82 | 67 | 26 | 1.4 | 69 |
| Sept. | 16 | 56 | 14 | 0.9 | 36 | 38 | 12 | 0.8 | 58 |
| ALL | 182 | 28 | 103 | 0.6 | 59 | 40 | 175 | 1.0 | 70 |

TABLE 9

*Seasonal Abundance and Sex Ratio of Fleas from Cynomys gunnisoni at St. Michael's College*
*March–September, 1952*

| Month | Hosts Examined | *Opisocrostis hirsutus* Per cent Hosts Infested | Fleas Collected | Fleas Average per Host | Per cent Female Fleas | *Opisocrostis tuberculatus* Per cent Hosts Infested | Fleas Collected | Fleas Average per Host | Per cent Female Fleas |
|---|---|---|---|---|---|---|---|---|---|
| Mar. | 13 | 92 | 103 | 7.9 | 53 | 92 | 110 | 8.5 | 51 |
| Apr. | 25 | 64 | 118 | 4.7 | 66 | 84 | 237 | 9.5 | 58 |
| May | 23 | 22 | 10 | 0.4 | 50 | 13 | 5 | 0.2 | 80 |
| June | 13 | 8 | 1 | 0.1 | 100 | 0 | 0 | 0 | .. |
| July | 11 | 55 | 34 | 3.1 | 76 | 0 | 0 | 0 | .. |
| Aug. | 17 | 41 | 29 | 1.7 | 59 | 0 | 0 | 0 | .. |
| Sept. | 11 | 100 | 166 | 15.1 | 49 | 0 | 0 | 0 | .. |
| ALL | 113 | 51 | 461 | 4.1 | 57 | 32 | 352 | 3.1 | 56 |

39. *Opisocrostis tuberculatus cynomuris* Jellison*[1]

Local distribution. All records from St. Michael's College in the Upper Sonoran Zone. Although present, *C. gunnisoni* was not taken at the Santa Fe Airport.

[1] Macchiavello (1954) lists *O. tuberculatus* (subspecies not given) as a plague vector.

Host record:

| | | | | |
|---|---|---|---|---|
| *Cynomys gunnisoni* ..... | 134; | 19 ; | 2.6 ; | 13.5 |

Seasonal distribution. Limited records (Table 9) indicate the flea occurs on *C. gunnisoni* only during March, April, and May. The host was in hibernation from October through February and no records of the flea were obtained during this period. Gravid females were found in March and April, the only months when females were examined.

Sex ratio. Females were predominant in each month of collection; of 352 fleas, 56 per cent were females (Table 9).

40. *Orchopeas caedens caedens* (Jordan)

Host records. None.

Nest records. Of four *Tamiasciurus hudsonicus* nests collected from the Canadian Zone on October 6, 1953, three nests were infested with a total of 12 male and four female fleas. Two of the four females were gravid. The same three nests contained 35 flea larvae that were held in the laboratory; a single male of *O. caedens* emerged sometime prior to November 9.

41. *Orchopeas leucopus* (Baker)

Local distribution. Recorded from Santa Fe Airport and other localities in the Upper Sonoran Zone.

Host records:

| | | | | |
|---|---|---|---|---|
| *Citellus spilosoma* ..... | 326; | 1—; | 0.1—; | 1.0 |
| *Citellus variegatus* ..... | 9; | 11 ; | 0.1 ; | 1.1 |
| *Perognathus flavus* ..... | 989; | 1—; | 0.1—; | 1.0 |
| *Dipodomys ordii* ..... | 2,232; | 1—; | 0.1—; | 1.0 |
| *Reithrodontomys megalotis*.. | 130; | 21 ; | 0.5 ; | 2.2 |
| *Peromyscus maniculatus* ... | 445; | 17 ; | 0.3 ; | 1.8 |
| *Peromyscus leucopus* ..... | 285; | 34 ; | 0.8 ; | 2.2 |
| *Peromyscus boylei* ..... | 27; | 7 ; | 0.1—; | 1.0 |
| *Peromyscus truei* ..... | 662; | 39 ; | 0.9 ; | 2.2 |
| *Onychomys leucogaster* .... | 405; | 1 ; | 0.1—; | 1.2 |
| *Neotoma micropus* ..... | 579; | 1—; | 0.1—; | 5.0 |
| *Neotoma albigula*..... | 981; | 1—; | 0.1—; | 1.0 |

Nest records. Two *P. truei* nests were infested; an April collection had 16 male and 16 female fleas, a June collection contained only two females.

Seasonal distribution. The flea reached greatest abundance from April through August and least abundance from November through February. It was taken from *P. truei* (Table 6) and *P. maniculatus* (Table 7) during all months of the year but was absent from small numbers of *P. leucopus* (Table 8) examined in December and January. Gravid females were collected in all months except November when only seven females were examined.

Sex ratio. Females predominated in the annual totals from each of the three hosts (Tables 6, 7, 8). Excluding several instances where less than 10 fleas were examined, females predominated in monthly collections from each of the three hosts except the April, 1953, collection from *P. truei*.

Several pools of this species, collected near Lamy in May, were infected with *P. pestis* (Holdenried and Morlan, in press).

42. *Orchapeas neotomae* Augustson

Local distribution. Upper Soronan and Transition Zones; absent from the extensive Santa Fe Airport collections.

Host records:

| | | | | |
|---|---|---|---|---|
| *Neotoma micropus* ..... | 579; | 1—; | 0.1—; | 1.7 |
| *Neotoma albigula* ..... | 981; | 1 ; | 0.1—; | 2.1 |
| *Neotoma mexicana* ..... | 9; | 44 ; | 2.0 ; | 5.0 |
| *Neotoma cinerea* ..... | 2; | 50 ; | 0.5 ; | 1.0 |

Nest records. A single male was removed from a *N. albigula* nest collected in November, 1951.

Seasonal distribution. Small collections were made during the months from May through November.

Sex ratio. Of 42 fleas, 48 per cent were females.

A pool of four fleas, collected from *N. albigula* taken near Lamy on June 11, 1953, was infected with *P. pestis* (Holdenried and Morlan, in press).

43. *Orchopeas sexdentatus* (Baker)*

Local distribution. Upper Sonoran (including Santa Fe Airport) and Transition Zones.

Host records:

| | | | | |
|---|---|---|---|---|
| *Sylvilagus audubonii* ..... | 170; | 2 ; | 0.1 ; | 6.3 |
| *Dipodomys spectabilis* ..... | 1,128; | 1—; | 0.1—; | 1.0 |
| *Peromyscus maniculatus*.... | 445; | 1—; | 0.1—; | 1.0 |
| *Peromyscus truei* ..... | 662; | 1—; | 0.1—; | 1.2 |
| *Onychomys leucogaster* .... | 405; | 1—; | 0.1—; | 1.0 |
| *Neotoma micropus* ..... | 579; | 78 ; | 6.6 ; | 8.4 |
| *Neotoma albigula* ..... | 981; | 31 ; | 0.8 ; | 2.6 |
| *Neotoma mexicana* ..... | 9; | 11 ; | 0.1 ; | 1.0 |
| *Neotoma cinerea* ..... | 2; | 100 ; | 10.0 ; | 10.0 |

Nest records. Eight of 10 *N. micropus* nests were infested with a total of 97 male and 151 female fleas. Six of 28 *N. albigula* nests were infested with a total of only 11 male and 12 female fleas. Nests with *O. sexdentatus* were found in August and October through April. About 500 flea larvae collected April 23, 1952, from a *N. micropus* nest produced four male and two female adults of *O. sexdentatus* by June 12. Ten, of over 1,000 larvae in a *N. micropus* nest taken March 25, 1952, were saved for rearing; one pupated March 29 and an adult female of *O. sexdentatus* emerged on April 9.

Seasonal distribution. Abundance, as shown on *N. micropus* (Table 10) and on *N. albigula* (Table 11), displayed marked seasonal variation with summer (May–August) maxima and winter (December–February) minima. The greater proportion of females examined in each month of the year were gravid.

Sex ratio. On both *N. micropus* and *N. albigula* (Tables 10 and 11), females comprised 58 to 61 per cent of the yearly collections. Males predominated in collections of some months from September through January but records from the two hosts and years were not consistent.

TABLE 10

*Seasonal Abundance and Sex Ratio of Orchopeas sexdentatus from Neotoma micropus at Santa Fe Airport*

| Month | Year | Hosts Examined | Per cent Hosts Infested | Fleas Collected | Fleas Average per Host | Per cent Female Fleas |
|---|---|---|---|---|---|---|
| Oct. | 51 | 8 | 75 | 54 | 6.8 | 48 |
| | 52 | 33 | 70 | 123 | 3.7 | 53 |
| Nov. | 51 | 9 | 78 | 29 | 3.2 | 83 |
| | 52 | 24 | 58 | 74 | 3.1 | 59 |
| Dec. | 51 | 9 | 67 | 17 | 1.9 | 53 |
| | 52 | 24 | 58 | 48 | 2.0 | 56 |
| Jan. | 52 | 11 | 64 | 22 | 2.0 | 73 |
| | 53 | 19 | 32 | 16 | 0.8 | 44 |
| Feb. | 52 | 20 | 55 | 30 | 1.5 | 63 |
| | 53 | 17 | 76 | 37 | 2.2 | 59 |
| Mar. | 52 | 16 | 63 | 33 | 2.1 | 61 |
| | 53 | 21 | 71 | 46 | 2.2 | 67 |
| Apr. | 52 | 30 | 87 | 145 | 4.8 | 63 |
| | 53 | 15 | 80 | 68 | 4.5 | 65 |
| May | 52 | 39 | 85 | 343 | 8.8 | 58 |
| | 53 | 10 | 90 | 34 | 3.4 | 79 |
| June | 52 | 28 | 93 | 449 | 16.0 | 61 |
| | 53 | 17 | 100 | 297 | 17.5 | 61 |
| July | 52 | 43 | 95 | 481 | 11.2 | 64 |
| | 53 | 15 | 93 | 267 | 17.8 | 60 |
| Aug. | 52 | 38 | 95 | 521 | 13.7 | 56 |
| | 53 | 15 | 93 | 60 | 4.0 | 65 |
| Sept. | 52 | 40 | 98 | 326 | 8.2 | 53 |
| | 53 | 15 | 87 | 63 | 4.2 | 46 |
| ALL | 1st | 291 | 85 | 2450 | 8.4 | 59 |
| | 2nd | 225 | 73 | 1133 | 5.0 | 60 |

44. *Oropsylla idahoensis* (Baker)

| | | | | |
|---|---|---|---|---|
| *Citelus lateralis* ..... | 29; | 10 ; | 0.1 ; | 3.0 |
| *Eutamias quadrivittatus* .... | 88; | 1 ; | 0.1—; | 1.0 |

Only two male and two female fleas were collected during August and September in the Transition Zone.

45. *Tarsopsylla coloradensis* (Baker)

Host records. None.

Nest records. Each of four *Tamiasciurus hudsonicus* nests, taken October 6, 1953, in the Canadian Zone, were infested. A total of 13 male and 12 female fleas were collected; two of the 12 females were gravid.

46. *Thrassis campestris* Prince

Local distribution. Santa Fe Airport and other nearby locations in the Upper Sonoran Zone.

TABLE 11

*Seasonal Abundance and Sex Ratio of Orchopeas sexdentatus from Neotoma ilbigula at Santa Fe Airport*

| Month | Year | Hosts Examined | Per cent Hosts Infested | Fleas Collected | Fleas Average per Host | Per cent Female Fleas |
|---|---|---|---|---|---|---|
| Oct. | 51 | 24 | 38 | 16 | 0.7 | 63 |
| | 52 | 82 | 24 | 27 | 0.3 | 63 |
| Nov. | 51 | 11 | 10 | 1 | 0.1 | 0 |
| | 52 | 25 | 12 | 4 | 0.2 | 75 |
| Dec. | 51 | 7 | 14 | 1 | 0.1 | 0 |
| | 52 | 25 | 12 | 5 | 0.2 | 40 |
| Jan. | 52 | 19 | 0 | 0 | 0 | .. |
| | 53 | 25 | 8 | 2 | 0.1 | 50 |
| Feb. | 52 | 18 | 33 | 9 | 0.5 | 56 |
| | 53 | 20 | 25 | 10 | 0.5 | 40 |
| Mar. | 52 | 17 | 24 | 6 | 0.4 | 67 |
| | 53 | 26 | 19 | 6 | 0.2 | 67 |
| Apr. | 52 | 34 | 53 | 42 | 1.2 | 57 |
| | 53 | 15 | 40 | 13 | 0.9 | 69 |
| May | 52 | 41 | 73 | 80 | 2.0 | 60 |
| | 53 | 12 | 50 | 15 | 1.2 | 60 |
| June | 52 | 58 | 60 | 115 | 2.0 | 59 |
| | 53 | 15 | 80 | 55 | 3.7 | 62 |
| July | 52 | 66 | 62 | 125 | 1.9 | 58 |
| | 53 | 15 | 53 | 30 | 2.0 | 60 |
| Aug. | 52 | 59 | 49 | 61 | 1.0 | 51 |
| | 53 | 15 | 33 | 17 | 1.1 | 59 |
| Sept. | 52 | 73 | 38 | 60 | 0.8 | 62 |
| | 53 | 15 | 27 | 15 | 1.0 | 67 |
| ALL | 1st | 427 | 47 | 516 | 1.2 | 58 |
| | 2nd | 290 | 27 | 199 | 0.7 | 61 |

Host records:

| | | | | |
|---|---|---|---|---|
| *Dipodomys spectabilis* ..... | 1,128; | 3 ; | 0.1—; | 1.7 |
| *Dipodomys ordii* ..... | 2,232; | 3 ; | 0.1—; | 1.3 |
| *Peromyscus maniculatus*.... | 445; | 1—; | 0.1—; | 1.0 |
| *Peromyscus leucopus* ..... | 285; | 1 ; | 0.1—; | 1.4 |
| *Peromyscus truei* ..... | 662; | 1—; | 0.1—; | 1.0 |
| *Onychomys leucogaster* .... | 405; | 2 ; | 0.1—; | 1.1 |
| *Neotoma micropus* ..... | 579; | 1—; | 0.1—; | 1.2 |
| *Neotoma albigula* ..... | 981; | 1—; | 0.1—; | 1.2 |

Nest records. A single male was removed from a *D. spectabilis* nest; another male from a *D. ordii* nest. Both nests were collected in February.

Seasonal distribution. Of 174 fleas on all hosts, 98 were collected in October, 45 in November, none from April through August. Gravid females were observed from October through December.

Sex ratio. Of 174 fleas, 62 per cent were females.

47. *Thrassis fotus* (Jordan)*

*Citellus variegatus* ..... 29; 3 ; 0.1—; 1.0

A single female was taken from the Transition Zone in August.

48. *Thrassis pansus* (Jordan)

Local distribution. Records mostly from Santa Fe Airport; all from Upper Sonoran Zone.

Host records:

| | | | | |
|---|---|---|---|---|
| *Cymomys gunnisoni* ..... | 134; | 2 ; | 0.1—; | 1.0 |
| *Citellus spilosoma* ..... | 326; | 54 ; | 2.2 ; | 4.1 |
| *Dipodomys spectabilis* ..... | 1,128; | 1 ; | 0.1—; | 1.5 |
| *Dipodomys ordii* ..... | 2,232; | 1—; | 0.1—; | 1.0 |
| *Reithrodontomys megalotis*.. | 130; | 1—; | 0.1—; | 4.0 |
| *Peromyscus maniculatus* .... | 445; | 1—; | 0.1—; | 1.0 |
| *Peromyscus leucopus* ..... | 285; | 1—; | 0.1—; | 1.0 |
| *Onychomys leucogaster* .... | 405; | 10 ; | 0.2 ; | 1.7 |
| *Neotoma micropus* .... | 579; | 1—; | 0.1—; | 1.2 |
| *Neotoma albigula* ..... | 981; | 1—; | 0.1—; | 1.0 |

Seasonal distribution. *C. spilosoma*, being in hibernation, was not taken from December through March. A few fleas were taken from *O. leucogaster* in March and December; from *D. ordii* in December. On C. *spilosoma* (Table 12) major trends in abundance were not clearly apparent; however,

TABLE 12

*Seasonal Abundance and Sex Ratio of Thrassis pansus from Citellus spilosema at Santa Fe Airport*

| Month | Year | Hosts Examined | Per cent Hosts Infested | Fleas Collected | Fleas Average per Host | Per cent Female Fleas |
|---|---|---|---|---|---|---|
| Oct. | 51 | 22 | 55 | 111 | 5.0 | 56 |
| | 52 | 14 | 64 | 26 | 1.9 | 62 |
| Nov. | 51 | 9 | 56 | 16 | 1.8 | 63 |
| | 52 | 6 | 83 | 34 | 5.7 | 79 |
| Apr. | 52 | 34 | 71 | 116 | 3.4 | 68 |
| | 53 | 1 | 100 | 1 | 1.0 | 100 |
| May | 52 | 46 | 59 | 80 | 1.7 | 73 |
| | 53 | 4 | 50 | 11 | 2.8 | 73 |
| June | 52 | 26 | 39 | 42 | 1.6 | 69 |
| | 53 | 8 | 50 | 20 | 2.5 | 60 |
| July | 52 | 48 | 73 | 140 | 2.9 | 64 |
| | 53 | 9 | 67 | 18 | 2.0 | 89 |
| Aug. | 52 | 32 | 44 | 39 | 1.2 | 69 |
| | 53 | 7 | 14 | 5 | 0.7 | 20 |
| Sept. | 52 | 16 | 25 | 15 | 0.9 | 60 |
| | 53 | 8 | 88 | 16 | 2.0 | 63 |
| ALL | 1st | 233 | 56 | 559 | 2.4 | 65 |
| | 2nd | 57 | 61 | 131 | 2.3 | 69 |

the two years' observations indicate more frequent occurrence in spring and fall. In each month from April through December, the majority of females examined were gravid.

Sex ratio. Of 690 fleas taken on *C. spilosoma* at the Santa Fe Airport in a two-year period, 66 per cent were females; females predominated in all months except August, 1953, when only five fleas were collected (Table 12). In each year during September and October, the proportion of females was less than the annual ratio.

49. *Thrassis stanfordi* Wagner

| | | | | |
|---|---|---|---|---|
| *Marmota flaviventris* ..... | 6; | 100 ; | 7.3 ; | 7.3 |

The collection contained 22 male and 22 female fleas taken from the Canadian Zone in August.

50. *Peromyscopsylla draco* Hopkins

Local distribution. Upper Sonoran Zone; all but one flea from Santa Fe Airport.

Host records:

| | | | | |
|---|---|---|---|---|
| *Reithrodontomys megalotis*.. | 130; | 7 ; | 0.1—; | 1.2 |
| *Peromyscus maniculatus* ... | 445; | 1—; | 0.1—; | 1.2 |
| *Peromyscus leucopus* .... | 285; | 1—; | 0.1—; | 1.0 |
| *Peromyscus truei* ..... | 662; | 1 ; | 0.1—; | 1.0 |
| *Neotoma micropus* .... | 579; | 1—; | 0.1—; | 1.0 |

Seasonal distribution. A single female flea was collected in August; all others were collected from October through March; 18 of 29 fleas were obtained in October and November. Both of the two females examined in January and August were gravid.

Sex ratio. Of 29 fleas, 69 per cent were females.

51. *Peromyscopsylla hamifer vigens* (Jordan)

| | | | | |
|---|---|---|---|---|
| *Microtus longicaudus* ..... | 41; | 15 ; | 0.2 ; | 1.7 |

During October and November five male and five female fleas were collecter from the Canadian Zone.

52. *Peromyscopsylla hesperomys adelpha* (Rothschild)

Local distribution. Upper Sonoran (including Santa Fe Airport) and Transition Zones.

Host records:

| | | | | |
|---|---|---|---|---|
| *Eutamias quadrivittatus* .... | 88; | 1 ; | 0.1—; | 1.0 |
| *Dipodomys ordii* ..... | 2,232; | 1—; | 0.1—; | 3.0 |
| *Reithrodontomys megalotis*.. | 130; | 1—; | 0.1—; | 1.0 |
| *Peromyscus maniculatus*.... | 445; | 1—; | 0.1—; | 1.0 |
| *Peromyscus leucopus* ..... | 285; | 1 ; | 0.1—; | 2.0 |
| *Peromyscus truei* ..... | 662; | 2 ; | 0.1—; | 1.3 |
| *Peromyscus nasutus*..... | 21; | 5 ; | 0.1—; | 2.0 |

Nest records. Three females were removed from a *N. microtus* nest taken in November, 1953.

Seasonal distribution. Collections were in all months except April, August, September, and December. Of 11 females examined, seven, collected in February, May, October, and November were gravid.

Sex ratio. Of 40 fleas, 56 per cent were females.

Each of three fleas, collected from *P. truei*, *P. maniculatus*, and *P. leucopus* taken near Lamy in May, were infected with *P. pestis* (Holdenried and Morlan, in press).

53. *Peromyscopsylla selenis* (Rothschild)

*Microtus longicaudus* ..... 41; 7 ; 0.1—; 1.0

Two male fleas and one female were collected during August in the Transition and Canadian Zones.

*Discussion.* Thompson (1954) quotes an apt remark of an unnamed biologist as follows: "In describing many distributional oddities such terms as 'rare' and 'endemic' could be applied more appropriately to the collector than to the species studied." It should be emphasized that the preceding records were primarily obtained through an intensive two year study at the Santa Fe Municipal Airport. In the admittedly biased sample of Santa Fe County mammal fleas, 13 species accounted for over 95 per cent of the 26,250 fleas that were determined. In order of decreasing frequency, the fleas collected were: *M. rectus*, *O. sexdentatus*, *M. nidi*, *H. affinis*, *C. inaequalis*, *O. leucopus*, *M. wagneri*, *T. pansus*, *O. hirsutus*, *M. exilis*, *M. parkeri*, *O. tuberculatus*, and *M. jamesoni*. A fairly reliable idea of degree of host interchange has been indicated for each of these species; detailed data on seasonal abundance and sex ratio were recorded in tables 1 through 12. Additional studies in other areas and in other years with more stress on the fauna of rodent nests should help to fill the numerous gaps in our knowledge. Until sufficient information is obtained to distinguish the important transmittors of disease from the long list of potential vectors, we should continue to accumulate data on even the infrequently encountered species of ectoparasites.

Only a few carnivores were collected. They may, of course, act as occasional disseminators of infective fleas but our records are inadequate to indicate frequency of carnivore infestation by rodent fleas. The fleas of birds and domestic animals were not investigated. Limited personal observations show fleas to be rare or absent on cats and dogs of Santa Fe. Domestic rats (*Rattus* spp.) are not known to occur in the study area. Twenty

to 30 house mice *(Mus musculus)* were examined without finding any ectoparasites.

Observations were insufficient to definitely define limits of local distribution for Santa Fe County mammal fleas but a great deal of contributory information was obtained. The most intensive collections from the Santa Fe Airport resulted in records of 22 species of fleas; all but one *(O. dentatus)* also were collected elsewhere in the Upper Sonoran Zone. Additional collections from rabbits would undoubtedly extend the known range of *O. dentatus*. A total of 38 species of fleas were recorded from the Upper Sonoran Zone; 16 were absent from Airport collections. Four of the 16 species *(D. montanus, F. ignota, O. hirsutus,* and *O. tuberculatus)* are normal parasites of hosts which occurred but were not collected at the Airport. The usual hosts of *M. eumolpi* and *M. vison* do not occur at the Airport. Reasons for the absence of the 10 other species of fleas from the Airport were not evident, but absence probably is due to habitat requirements which are presently unknown.

There was considerable overlap of flea species between Life Zones. Records of species that were restricted to a single Zone included 21 of the 38 from the Upper Sonoran, three of the 19 from the Transition, and nine of the 15 from the Canadian Zone. Further collections, especially in the Transition and Canadian Zones, would extend the known local distribution of at least part of the flea species.

In a study of the oriental rat flea, *Xenopsylla cheopis* (Rothschild), recovered from live rats, Cole (1945) showed a significant tendency for males to outnumber females on days with a high mean temperature. He suggested that the sexes of *X. cheopis* occur in equal numbers in nature and that a count of females may bear a more stable relationship to the total population than a count of total fleas. In southwestern Georgia, Morlan and Utterback (1952) reported a similar tendency for an increased proportion of male *X. cheopis* with increased mean temperature but found no regular association between sex ratio and mean temperature for the mouse flea, *Leptopsylla segnis* (Schönherr); in general, females of both species were predominant with the percentage of female fleas higher for *L. segnis* than for *X. cheopis*.

As shown in the present report, there was a general tendency for females to outnumber males in collections of fleas from western rodents. In every species where at least 50 fleas were examined, females were in the majority. Observations of monthly changes in sex ratio for 13 frequently collected species of fleas (Tables 1–12) were not especially informative. For example, males of *C. inaequalis* were predominant in July and August collection of 1953 but not in the same months of 1952; whereas males of *M. jamesoni* were predominant in July 1952 but not in July 1953. In certain species *(C. inaequalis, H. affinis, O. sexdentatus)* there appeared to be a tendency for the proportion of females to decrease during the first or second months following the peak of seasonal abundance. It is suggested that the observed changes in sex ratio may be primarily associated with seasonal changes in reproductive activity which, in turn, are probably influenced by climatic conditions.

Males were predominant in a few small collections from rodent nests, however, such collections were too limited to provide much conclusive data. Females of *A. nudatus* from rodent nests outnumbered males in all months of the year except for small collections in December and February.

There appeared to be some evidence that male fleas predominate on secondary hosts, *e.g.*, *M. nidi* from *D. ordii* and *M. parkeri* from *O. leucogaster*. The observations can be explained only with more detailed knowledge of host-parasite relationships.

A comparison of general flea infestation on some of the more commonly collected mammals is shown in the following list; the first number after the name of each mammal indicates the number of individuals examined (or re-examined), the second number is the average number of fleas per examination, and the third is the number of flea species:

| | | | |
|---|---|---|---|
| *S. audubonii* | 170; | 33.0; | 7 |
| *C. spilosoma* | 326; | 2.2; | 9 |
| *C. gunnisoni* | 134; | 7.0; | 6 |
| *P. flavus* | 989; | 0.2; | 8 |
| *D. spectabilis* | 1,128; | 8.5; | 10 |
| *D. ordii* | 2,232; | 0.4; | 10 |
| *O. leucogaster* | 405; | 1.9; | 13 |
| *R. megalotis* | 130; | 0.8; | 8 |
| *P. maniculatus* | 445; | 0.8; | 19 |

| | | | |
|---|---|---|---|
| *P. leucopus* | 285; | 1.5; | 12 |
| *P. truei* | 662; | 1.9; | 18 |
| *N. micropus* | 579; | 6.7; | 15 |
| *N. albigula* | 981; | 1.1; | 23 |

To some extent, the number of species of ectoparasites that may be collected from a given species of mammal tends to increase with an increasing number of individuals examined due to the greater possibility for chance infestations. However, it appears, from data shown above, that certain mammals are naturally infested with larger numbers of flea species as well as with larger average numbers of fleas. The large average number of fleas (33.0) found on *S. audubonii* do not necessarily indicate an increased opportunity for spread of disease but could so indicate if the number consisted largely of efficient vectors. The large number of flea species (23) recovered from *N. albigula* might indicate a wide association with other mammals conducive to spread of disease but does not take into account the unknown frequency with which fleas of a single species may transfer from one animal to another. It is interesting to note, for example, that flea collections from *N. albigula* average 1.1 compared to 6.7 from *N. micropus*, however, the meaning of these differences, in terms of disease transmission, is not known.

Flea-host relationships are in part dependent upon the particular assemblage of rodents in any specific locality; *e.g.*, *T. pansus* occurs on *D. spectabilis* when the rats' habitat overlaps that of the primary host, *C. spilosoma*. The finding of a given species on an abnormal host may indicate the presence of the primary host. Moreover, the degree of flea interchange between hosts may contribute circumstantial evidence of rodent habits. For example, *D. ordii* appeared to be the primary host of *M. parkeri;* the flea was more frequently found on *N. micropus* than *N. albigula* which may indicate that *N. micropus* were usually more closely associated with *D. ordii* than were *N. albigula*. As comprehensive knowledge of host-parasite relationships is attained, equal or reduced time expended in trapping activities should yield greater returns in evaluation of conditions affecting spread of disease.

Economic control of rodent-borne disease must depend upon more knowledge than is presently available. Until circumstances

permit continuity in biological research, significant advances will be most fortuitous. The foregoing observations on the biology of fleas in Santa Fe County fall short of a comprehensive picture but when added to information from future studies may provide useful contributions to our understanding of the rodent-ectoparasite-disease complex.

## *Summary*

From July, 1951 to February, 1954, over 26,000 fleas of 53 species were collected in Santa Fe County, New Mexico. Data on host relationships, sex ratio, local distribution, and seasonal abundance were obtained by examination of 8,784 mammals and 66 rodent nests. Comprehensive seasonal data were obtained for a two-year period, October, 1951, through September, 1953, by weekly examination of live mammals from the Santa Fe Municipal Airport.

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# THE PROLONGATION OF MURINE TYPHUS RICKETTSEMIA BY CORTISONE*

MORRIS POLLARD AND BROTHER RAPHAEL WILSON

The epidemiological pattern of murine typhus depends on environmental conditions which are conducive to rodent and ectoparasite propagation and on the introduction of the rickettsial agent into such a community. When infected fleas (*Xenopsylla cheopis*) deposit feces on the skin of a susceptible rat, the rat becomes infected. In the pathogenesis of murine typhus the rat develops an asymptomatic transient rickettsemia, after which the agent recedes and its eventual residence in the brain tissue may last for months (Nicolle and Laigret, 1933; Lepine, 1933; Philip and Parker, 1938). When ectoparasites (*e.g.*, fleas) feed on rickettsemic animals, the agent invades the intestinal wall of the flea, multiplies there, and is excreted thereafter with feces. If such infected feces should contact the broken skin of a susceptible animal, the agent invades and infects the new host and the pathogenic pattern, as described above, is repeated. The infective flea retains the typhus agent for the rest of its life but does not perpetuate the infective agent through transovarial passage to its offspring (Blanc, Martin, and Baltazard, 1944; Cedar *et al.*, 1931). The perpetuation of infection within the community would therefore be facilitated either by frequent additions of susceptible rodents or by an occasional prolonged rickettsemia among the infected rodents.

The purpose of this brief report is to describe the effect of cortisone on the rickettsemic pattern of the infected rat. It has been observed that cortisone appears to reduce the resistance of the host so as to render him either more susceptible to infection (Kilbourne and Horsfall, 1951; Whitmire and Downs, 1954) or to so alter its course in the host as to influence the epidemiological pattern of the disease (Kass *et al.*, 1950; Shwartzman and Aronson, 1953).

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*Methods.* The Wilmington strain of murine typhus in yolk sac tissue was inoculated into 4 groups of white rats (0.1 cc. S.C.). Two groups of rats had received 20 mg. of cortisone[1] every 7 days for the preceding 2 weeks; the other two groups received no cortisone. In the cortisone-inoculated groups, the drug was continued at weekly intervals for the next 4 weeks. The rats were bled daily from the end of the incised tail into heparin, and the pooled blood from each group was inoculated intraperitoneally into two rats for one week. Approximately 0.5 ml. of blood was collected from each rat every day. Two new rats were inoculated each week with pooled blood from each of the donor groups. Two weeks following the last blood inoculations, each rat was etherized and exsanguinated from the heart, and each serum was tested for complement fixing antibodies with specific murine typhus antigen prepared from infected yolk sac. In this manner the blood-inoculated rats functioned as indicators of the rickettsemic pattern in the donor rats, and by this means its duration could be determined.

## *Results*

As indicated in table 1, blood from the cortisone-free rats was infective for 2 weeks; and for the 2 weeks thereafter, the typhus agent could not be demonstrated in their blood. The rats that received cortisone manifested a rickettsemia for at least 4 weeks. The experiment had been arbitrarily terminated at this point, so the maximum duration of blood infectivity under the influence of cortisone was not determined. There were no specific symptoms in any of the rats that might be attributable to typhus infection.

*Discussion and Summary.* Under "normal" conditions it appears that the typhus infected rat may infect blood-sucking ectoparasites for periods not exceeding 2 weeks after exposure to infection. Under the influence of cortisone this infectiousness of blood was prolonged to at least 4 weeks. Since fleas do not transmit the typhus agent to progeny transovarially then the prolongation of rodent rickettsemia may considerably influence the epidemiology of the disease and under the conditions here described the rat assumes a more secure reservoir status.

[1] Kindly provided by Shering Company.

It has been recorded by Philip and Parker (1938) that the rickettsemia in rodents may last as long as 1 month. Our results fail to demonstrate the agent in the peripheral blood of rats later than 2 weeks following inoculation. The prolongation of asymptomatic rickettsemia in rats with cortisone may simulate a physiological means by which the rat may exert a more sustained role in the dissemination of murine typhhus through blood sucking ectoparasites.

TABLE 1

*The Effect of Cortisone on Infectivity of Blood from Typhus-Infected Rats*

| Typhus infected rats | Rickettsemia—Days after inoculation | | | |
|---|---|---|---|---|
| | 0–7 | 8–14 | 15–21 | 22–28 |
| Without cortisone ..... | 3/4* | 4/7 | 0/3 | 0/5 |
| With cortisone ..... | 2/2 | 6/6 | 2/4 | 3/5 |

* Number of rats with positive C.F. test/number of indicator rats inoculated.

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# THE USE OF INERT, PLASTIC FABRICS AS ARTERIAL PROSTHESES*

EDGAR J. POTH, JOSEPH K. JOHNSON, AND JOHN H. CHILDERS

The failure of survival of arterial homografts and heterografts certainly should be expected since there is no evidence that any other heterologous or homologous tissues live when transplanted. These implanted vascular segments serve as a temporary conduit for the streaming blood and are eventually replaced by the tissues of the host. These replacing tissues are essentially fibrous connective tissues with a glistening inner lining of the conduit closely resembling the intima of the adjoining artery of the host. Arterial and venous autografts survive transplantation and it was shown by Parsons, Gerbode, and Cox (1952) that a vascular autograft covered by silver foil will survive to indicate that sufficient nutrition is supplied from the blood within the lumen of the transplant. The feasibiliy of using vascular grafts to replace injured or diseased segments of arteries was demonstrated by Carrel (1908) nearly half a century ago.

Autografts, except for small vessels, are not available practically. Creech, DeBakey, Self, and Halpert (1954) have shown heterografts to be undesirable because of the relatively rapid degenerative changes which occur. Homografts have been used quite extensively during the past five to six years. It is rather surprising how well these homografts have withstood the continuous, dilating action of the arterial blood pressure as demonstrated by segments implanted by Gross for correction of coarctation of the thoracic aorta. The number of cases which have gone as long as five years is quite small. The collection and preservation of suitable homografts is difficult, time consuming and expensive.

If a conduit, fabricated from an inert material, could be used instead of homografts, the procedure would be greatly simplified and could be more widely applied. Impervious, semirigid and rigid, plastic and metal tubes have been used to bridge across

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arterial defects caused by war injuries. These prostheses become occluded after a few days, but they apparently maintain sufficient circulation until collateral vessels can develop.

Recently, a fundamental observation has been made. The diseased intima can be stripped out of an occluded artery with reestablishment of blood flow through the vessel without immediate thrombosis occurring even though anticoagulants are not used.

Voorhees, Jaretzki, and Blakemore (1952) and Blakemore and Voorhees (1954) successfully employed tubes of an inert woven fabric (Vinyon "N") to bridge defects in the abdominal aorta. Such fabric tubes have many inherent advantages over tubes of impervious materials. They are thin, flexible and moderately elastic, and they can be readily sewed to form tubes of the exact dimensions of the vessel which they are to replace. Moreover, it can be shown by histological study that the fabric is soon invaded by fibroblasts and capillaries in much the same manner as the arterial homograft is revascularized and eventually replaced by fibrous tissue. The thin mural thrombus which forms over the inner surface of the fabric tube is rapidly organized, and, in a matter of 30 to 60 days, is replaced by fibrous tissue which has a smooth surface simulating endothelium. The fabric prosthesis serves as a temporary bridge to reestablish the continuity of the vessel, provides a scaffolding for the eventual reformation of a living, smoothly-lined tube and adds strength to the wall of the vessel by the incorporation of the plastic fibers within the new fibrous wall of the vessel.

The purpose of this study is to evaluate several aspects of the problem. It is imperative that a practical technic for tailoring fabrics into accurately fitting prostheses be developed. Apparently, it is important for the implanted, inert prosthesis to be of essentially the same dimensions as the structure replaced. Attention is directed to the desirability of having the length of the prosthesis equal to the defect so as to prevent wrinkling of the material even though it may appear that the longitudinal tension is excessive. A frame to assist in the fabrication of these prostheses at the operating table under sterile conditions is illustrated in figure 1. It would be practically impossible to have a sufficiently large supply of prefabricated prostheses available to permit accurate matching as regards length, diameter and taper.

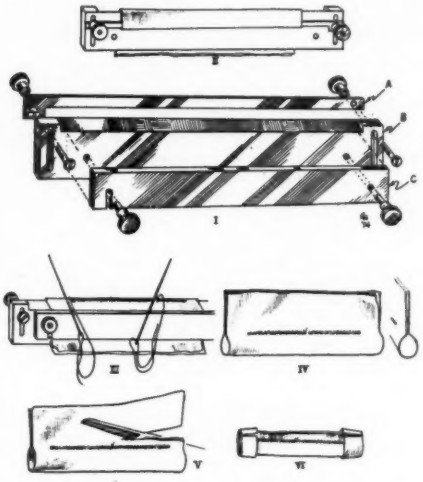



FIG. I. Adjustable frame designed to hold the fabric from which the prosthesis is made.

I. Detailed view of various parts of the apparatus. *A*. The adjustable bar over which the fabric is folded. This bar can be placed at the desired distance from the beveled edge of *B* at the distance required to give the size of tube needed. If the bar is placed parallel to the beveled edge, the prosthesis will have a uniform diameter from end to end. If bar, *A*, is placed in an oblique position to the beveled edge of *B*, then the prosthesis will taper from one end to the other. *C* can be clamped against *B* to hold the fabric in place as indicated in II for sewing as illustrated in III. A running stitch begins by being tied in the center, carried to each end. then returned to the center again and tied. IV shows the inner liner of the prosthesis upon removal from the frame. VI shows the finished inner liner, with cuffs turned back, ready for implantation. Since the prosthesis is made in a flattened state, the measured diameter of the recipient artery is multiplied by $\frac{\pi}{2}$ or 1.5 to obtain the "flattened" dimensions used in the apparatus.

(From Poth, Childers, Johnson and Guy: Vascular Replacements and Reconstruction Utilizing Inert Material. *The American Journal of Surgery* (In press).

The most suitable inert substance must be determined. Foreign-body tissue reaction must be minimal. The substance ultimately chosen must not lose its elasticity and must not fragment or decrease appreciably in tensile strength for a minimum of twenty years' burial in body tissue.

*Available Fabrics.* The number of combinations of materials and weaves used in the textile industry is myriad. A few defi-

nitions will be required before this facet of the subject can be discussed.

1) Denier: An arbitrary unit derived as follows: one gram of the smallest fibre of silk useful in making yarn was found to be 9000 meters long. The denier of the yarn, be it monofilament or multifilament, is the weight in grams of 9000 meters of the yarn. For example, a 50 denier yarn is one of which a piece 9000 meters long weighs 50 grams.

2) Size. The oils used on yarn to facilitate weaving. Sulfonated oils are incorporatel to assist in "boiling off."

3) Boiling Off. The cleansing process by which "size" is removed before dyeing, etc.

4) Greige Fabric. A fabric as it comes from the weaving machine containing "size."

5) Finishing Materials. Starches, gelatins, synthetic resins, etc. added to fabric to give it body.

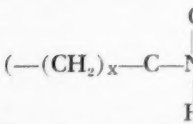
6) Weaves. A) Taffeta, B) Tricot, C) Satin, D) Net, E) Braids, and thousands of others.

The degree of "stretch" of a weave is determined by the elongation characteristics of the substance, the yarn, the weave, the finishing material and many other factors.

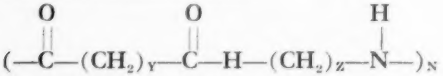
These fabrics may be woven from any number of yarns made of inert fibres. The fibres chosen for these studies are Vinyon "N," Nylon, Dacron and Orlon.

I) Vinyon "N." A trade name designating a copolymer of Vinyl chloride and acrylonitrile.

II) Nylon. A generic term for any long chain synthetic polymeric amide which has recurring amide groups as an integral part of the polymer chain. Note the similarity of this condensation with that of proteins.



Based on an amino acid.



Based on a dibasic acid and a diamine.

Obviously, this polymer is not a single compound. If the two extremes of the compounds condense to form high molecular weight, cyclic compounds, then the molecule would be highly stable with the following characteristics.

1) Very high flex life.
2) High strength with low weight.
3) Chemical resistance.
4) High extensibility and recovery from extensions.
5) Resistance to degradation by microorganisms.

III) Dacron. Trade name for a polymer involving the condensation of ethylene glycol and terephthalic acid,

$$(-C_2H_4-O-\overset{\overset{O}{\|}}{C}-C_6H_4-\overset{\overset{O}{\|}}{C}-O-)_N,$$

A polyester, having the following characteristics.

1) High flex life.
2) Strength.
3) Chemical resistance.
4) Resistance to degradation by microorganisms.

IV) Orlon. Trade name for a polymer of acrylonitrile, $(-CH_2-\underset{\underset{CN}{|}}{CH}-CH_2-\underset{\underset{CN}{|}}{CH}-CH_2-\underset{\underset{CN}{|}}{CH})_N$, having the following characteristics.

1) Good flex life.
2) Strength.
3) Chemical resistance.
4) Resistance to degradation by microorganisms.

All of these substances give promise of usefulness and may well be of equal value. Nylon has the desirable physical property of high extensibility and recovery as well as a chemical structure similar to the protein molecule. These properties may prove nylon to be superior.

The same "nylon" (66 polymer DuPont) should always be used for comparison or great care should be taken to designate the "nylon" being used. "Nylon" does not necessarily refer to the same formulation.

*Experimental Procedure.* Other workers have used a single thickness of fabric for the construction of the prosthesis. It is

reasoned that since these prostheses must remain intact for many years, reliance should not be placed on a single layer of fabric. A single broken yarn strand could spell disaster. Furthermore, if a fabric is studied closely under magnification, many defects will be found which are not evident on casual inspection. When multiple layered prostheses were sutured into place and blood introduced, it was found that the blood would frequently clot in the outer layers of the fabric first, and then clots would form between the concentric layers of the wall and result in occlusion of the lumen.

The technic was changed. A single layer of fabric was introduced and blood flow established. As soon as this inner liner became blood tight, additional layers of a similar or different fabric were added by wrapping.

Primarily, "Greige" nylon fabrics are used in this study. The "greige" fabrics are "boiled off." The fabric is boiled in water, rinsed in cold water, boiled for 40 minutes in water containing a detergent, and finally rinsed again repeatedly in clear water to ensure more nearly uniform material. A series of fabrics including 40 denier hosiery, 40 denier multifilament tricot, 15 denier monofilament tricot, a fine meshed net, and 70 denier taffeta with a thread count of $106 \times 76$ were used. At present, the 70 denier taffeta is used as the inner liner and the 15 denier monofilament tricot as the wrap around, adding two additional layers. Tricot is a knit fabric which stretches to a remarkable degree without alteration of shape. It is doubtful that this combination will ultimately prove to be the most satisfactory. Weaves using spun yarns and the so-called "Taslan" yarns offer intriguing possibilities.

The concept of the inner liner immediately suggests other possibilities. A vein autograft, encased in a supporting sheath, may well prove to be the most suitable procedure especially in the replacement of long segments of peripheral arteries of relatively small calibre. The inert, supporting sheath would determine the diameter of the transplant rather than the size of the vein autograft which might be somewhat smaller or larger than the replaced arterial segment. If a pervious supporting sheath is used over a homograft, the danger of late aneurysmal dilatation might well be eliminated. The elastic, inert fabric comprising the sheath

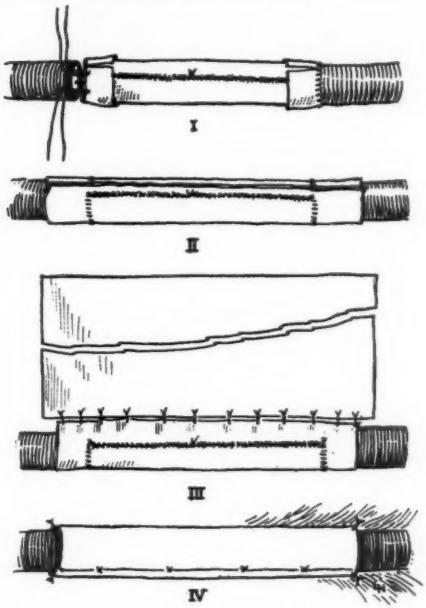



FIG. II. Illustrating the introduction of a simple prosthesis.

I. One end sewed in place with continuous, over and over stitch of 5–0 braided silk. The opposite end shows the everting, mattress traction sutures in place.

II. Showing the inner liner of 70 denier, 76 × 106 unfinished nylon in place with the cuffs extended over the lines of suture.

III. Showing the tricot weave of unfinished nylon "wrap-around" stitched to the longitudinal seam edge of the inner liner.

IV. Showing two layers of the "wrap-around" in place and stitched both longitudinally and the perivascular tissues at both ends.

(From Poth, Childers, Johnson and Guy: Vascular Replacements and Reconstruction Utilizing Inert Material. *The American Journal of Surgery* (In Press).

buried in the fibrous wall, would serve to function as the elastic tissue of the homegraft which would ultimately disappear.

The bifurcation of the abdominal aorta is a common site for arteriosclerotic occlusion and aneurysm formation. The fabrication of a smooth prosthesis of this rather intricate pattern presents a considerable problem. The simple procedure illustrated in figure III makes replacement possible with two simple, tapered tubes which can be tailored accurately to the required dimensions at the operating table. This same technic can be

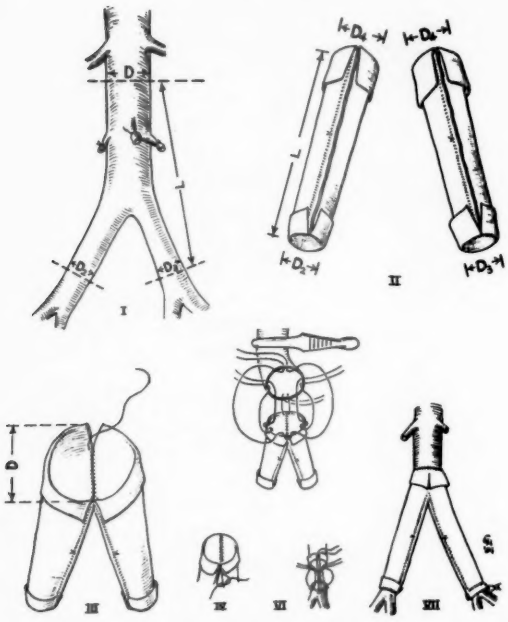



FIG. III. A schematic illustration of a technic for replacing the bifiguration of the aorta using two simple tubes.

I. D. the diameter of the aorta at the site of proximal resection. $D_2$ and $D_3$, the diameters of the right and left common iliacs at the levels of resection. L. the length of the resected segment. All of these dimensions are measured while the vessels are under pressure and before resection.

II. The two tubes which are to replace the bifurcation are fabricated at the operating table under aseptic conditions. $D_4$, the proximal diameter of these tubes, must be calculated. The circumference corresponding to $D_4$ is $\frac{\pi D}{2} + D$. It is sufficiently accurate to take the value 3 for $\pi$. The circumference of the tubes corresponding to $D_4$ would then equal 2.5 D, and $D_4 = \frac{2.5D}{\pi} = \frac{2.5D}{3} = 0.8$ D. But, these tubes are made "in the flat" in the frame illustrated in figure I and the measurement used in the frame is one-half the circumference or $F_4$, the measurement placed in the frame to correspond to $D_4$, equals $\frac{2.5\ D}{2}$ or 1.25 D. Likewise:

$$F_2 = \frac{\pi D_2}{2} = 3/2\ D_2 = 1.5\ D_2 \text{ and } F_3 = \frac{\pi D_3}{2} = 3/2\ D_3 = 1.5\ D_3$$

Thus, all of the measurements, L, $F_2$, $F_3$, and $F_4$, needed to make the tubes on the frame are determined.

III. These two tubes, which are somewhat tapered, are sewed together for the distance D using a running suture of 5–0 nylon. This suture begins on the outside, approximates the tubes for the distance D and then reverses on itself to introduce a second over and over stitch ending at and tied to the initial extremity of the suture as shown in Inset IV.

V. Shows the 4 everting stay sutures at the proximal line of anastomosis.

VI. Shows the 3 stay sutures at the distal line of anastomosis.

VII. Shows the completed 3 lines of anastomosis used to suture the inner liners in place. The bifurcation of the aorta has been replaced using two simple tubes tailored accurately to fit.

Simple geometric calculations show that the two replacement tubes have a greater cross-sectional area than the divided aorta when they return to cylindrical form. This relationship is not constant because a square of the radius enters into determination of the area of a circle.

applied to replacement of the bifurcation with homografts which would greatly simplify collection of these grafts. Smaller and healthier arteries obtained from younger individuals could be utilized. The two external iliacs and proximal femoral arteries could be used to replace the bifurcation of the aorta and the common, internal and external iliacs should it be desired to use a homograft. The abdominal aortae and bifurcations from two smaller individuals could be used to substitute for the abdominal aorta, bifurcation and common, internal and external iliacs.

Since impervious tubes of inert materials become encapsulated as foreign bodies and are not infiltrated by the tissues of the recipient, they can be considered unsatisfactory. One objectionable feature of pervious fabric tubes is the bleeding which occurs immediately upon restoration of blood flow. The blood shed depends upon the closeness of the weave and the thickness of the fabric. However, if the weave is too close and the thickness too great the vascularization of the intramural clot and ingrowth of fibroblasts may be retarded. Consequently, the weave must be sought which will most nearly fulfill these diverse requirements. Obviously, each individual fibre of the plastic material will be incased by the ingrowth of fibrous tissue and by collagen fibres if the space between the fibres is sufficient. Apparently, this encasement of the individual fibres of the implant results in minimal foreign body reaction. Microscopically, calcium deposition has been observed within a few weeks in an occasional nylon implant. Gross depositions of calcium demonstratable within 3 months by X-ray examination has occurred in orlon replacement of aortic segments in dogs. Preliminary observations would indicate a greater tendency for calcium deposition in orlon than in nylon implants. Attention should be directed towards this

TABLE 1

*Tabulation of Experimental Prosthetic Implantations in Dogs*

| Dog | Type of Prosthesis | Survival | Result of Aortagram | Findings at Autopsy |
|---|---|---|---|---|
| No. 1 | Double layer, taffeta inner-lined; tricot (multifilament) outer | 68 days | Not done | Died of food poisoning; prosthesis patent |
| No. 2 | Taffeta inner layer; monofilament tricot wrap-around | 90 days | Complete patency at 30 days | Sacrificed—prosthesis fully patent |
| No. 3 | Taffeta inner layer monofilament tricot wrap | 17 days | Not done | Died of pneumonia; fully patent prosthesis |
| No. 4 | Taffeta inner layer; monofilament tricot wrap-around | 40 days | Complete patency at 30 days | Sacrificed—prosthesis fully patent |
| No. 5 | Taffeta inner layer; monofilament tricot wrap-around | 45 days | Complete patency at 30 days | Sacrificed—prosthesis fully patent |
| No. 6 | Taffeta inner layer; monofilament tricot wrap-around | Living (107 d.p.o.) | Complete occlusion at 34 days | |
| No. 7 | Taffeta inner layer; monofilament tricot wrap-around | 31 days | Complete patency at 30 days | Sacrificed—prosthesis fully patent |
| No. 8 | Taffeta inner layer; monofilament tricot wrap-around | 30 days | Complete patency at 30 days | Sacrificed—prosthesis fully patent |
| No. 9 | Taffeta inner layer; monofilament tricot wrap-around | 39 days | Complete patency at 25 days | Sacrificed—prosthesis fully patent |
| No. 10 | Taffeta inner layer; monofilament tricot wrap-around | 33 days | Complete patency at 33 days | Died of pneumonia; prosthesis fully patent |
| No. 11 | Taffeta inner layer; monofilament tricot wrap-around | Living (102 d.p.o.) | Complete patency at 30 days | |
| No. 12 | Taffeta inner layer; monofilament tricot wrap-around | 40 days | Complete patency at 28 days | Sacrificed—prosthesis fully patent |
| No. 13 | Taffeta inner layer; monofilament tricot wrap-around | Living (98 d.p.o.) | Complete patency at 32 days | |
| No. 14 | Taffeta inner layer; moonfilament tricot wrap-around | Living (94 d.p.o.) | Complete patency at 27 days | |
| No. 15 | Taffeta inner layer; monofilament tricot wrap-around | 26 days | Complete patency at 26 days | Died from cord damage; prosthesis fully patent |
| No. 16 | Taffeta inner layer alone—no wrap | 54 days | Complete patency at 30 days | Died of infection; prosthesis fully patent |
| No. 17 | Taffeta inner layer alone—no wrap | Living (86 d.p.o.) | Complete patency at 30 days | |

TABLE 1—Continued
*Tabulation of Experimental Prosthetic Implantations in Dogs*

| Dog | Type of Prosthesis | Survival | Result of Aortagram | Findings at Autopsy |
|---|---|---|---|---|
| No. 18 | Taffeta alone—no wrap | Living (85 d.p.o.) | Complete patency at 30 days | |
| No. 19 | Taffeta inner layer plus tricot wrap | 36 days | Complete patency at 30 days | Died of infection; prosthesis fully patent |
| No. 20 | Taffeta inner layer plus tricot wrap | 16 days | Not done | Sacrificed—prosthesis patent |
| No. 21 | Nylon ribbon no cuffs or wrap | Living (65 d.p.o.) | Complete patency at 60 days | |
| No. 22 | Taffeta—no wrap | 35 days | Not done | Died of infection; prosthesis fully patent |
| No. 23 | Taffeta—bifurcation, no wrap | 14 days | Not done | Sacrificed—one limb of bifurcation occluded |
| No. 24 | Taffeta inner layer alone—no wrap | 16 days | Not done | Died of hemorrhage from insecurely ligated vessel —prosthesis patent |
| No. 25 | Taffeta bifurcation—no wrap | Living (40 d.p.o.) | Not done | |
| No. 26 | Taffeta bifurcation—no wrap | Living (38 d.p.o.) | Not done | |
| No. 27 | Taffeta bifurcation—no wrap | 4 days | Not done | Died of hemorrhage from insecurely ligated vessel. Both limbs fully patent |
| No. 28 | Taffeta bifurcation—no wrap | Living (36 d.p.o.) | Not done | |
| No. 29 | Taffeta bifurcation—no wrap | Living (35 d.p.o.) | Not done | |
| No. 30 | Taffeta bifurcation—no wrap | Living (34 d.p.o.) | Not done | |

possible complication which may well end in failure of all prostheses of this type within a span of a few years.

Thirty adult mongrel dogs were employed in this study, Table 1. The dogs were given a preoperative dose of sodium Nembutal intraperitoneally a few minutes before exposure of the external jugular vein to introduce a polyethylene cannula. Additional sodium Nembutal is given via this cannula in small doses to secure and maintain surgical anesthesia. An infusion of 5 per cent dextrose in water is started and continued throughout the operation. A total volume of less than 250 milliliters is given.

The operative technic follows. A broad area of the left flank is prepared by shaving, washing with soap and water, alcohol, ether, and finally 1:1000 Zephiran solution. Sterile drapes are sutured to the skin, and a long, oblique incision is made, begin-

ning at about the mid-inguinal region, curving over the crest of the ilium and extending upward to the tip of the 11th rib. The external oblique fascia is incised and the muscles transected at right angles. As soon as properitoneal fat is encountered, blunt dissection is used to reflect the peritoneum medially to expose the abdominal aorta. This approach gives excellent extraperitoneal exposure of the aorta from the level of the renal arteries to a point several centimeters below the trifurcation. The aorta is then freely mobilized by wiping away fat, lymphatics and the sympathetic plexus. Disruption of the larger lumbar and mesenteric lymphatic channels draining to the cysterna chyli often produces a troublesome flow of chylous fluid but leads to no untoward results. Mild shock, which is readily corrected by increasing the rate of flow of intravenous fluids momentarily, is observed occasionally when the plexus of nerves is stripped from the aorta.

The inferior mesenteric artery, the two lowermost pairs of lumbar arteries, and one or two small mesenteric branches are ligated and divided. Unshod bulldog clamps are applied above and below the segment to be excised. A measured segment of aorta, 3 to 5 centimeters in length, is excised after measuring the diameters above and below. The fat and loose adventitial connective tissue is trimmed away from the cut ends of the vessel. The inner liner of the prosthesis is tailored accurately to fit the defect. The proximal anastomosis is made first. The anastomosis to the vessel is made with 5–0 arterial silk on atraumatic needles. The most satisfactory technic of anastomosis is to suture between three or four stays. When each stay suture is reached by the continuous mattress stitch, the sutures are drawn taut to coapt closely the everted edges of the vessel and prosthesis. The running suture is then tied to the stay to minimize the tendency to purse-stringing. An over-and-over reinforcing stitch is used if doubt exists as to the security of the anastomosis. When this technic is meticulously executed, leakage at the anastomosis does not occur and the total blood loss is insignificant.

The studies of Lowenberg and Schumacker (1949) emphasize the vulnerability of the end-to-end anastomosis to disruption by traction. This observation supports use of the turned-down cuffs of the prosthesis to cover the adjacent ends of the artery, as these cuffs very soon become firmly fixed to the adventitia of the

vessel by coagulation. Schumacker and Lowenberg (1948) also compared the relative merits of various methods of suture and found that the continuous everting mattress suture offers the greatest security and the least tendency to leak.

On completion of the distal anastomosis, the lower clamp is removed, followed a second later by removal of the upper clamp. Blood loss through the pores of the cloth or leakage at the anastomosis is virtually absent if the technics recommended above are strictly followed. When the prosthesis is fully distended and pulsating, and it is determined that there is no narrowing of the line of anastomosis at either end, the cuffs of the tubes are turned over the ends of the host vessels and two or three interrupted sutures are placed at each end to secure them to the adventitia. Any blood clots remaining in the operative field are flushed out with warm saline, and the extra layers of tricot fabric are slipped under the prosthesis to extend just beyond the cuffs, stitched to the longitudinal seam of the inner liner and molded snugly into place. A few stitches at each end of the wrap-around anchor it under slight tension to the perivascular tissues. The prosthesis is sponged dry and then moistened with blood which is allowed to clot within the interstices of the fabric. The wound is closed in layers after all clots and debris are removed and the prosthesis cleaned of any extraneous blood clot. Antibiotics and anticoagulants are not administered.

*Postoperative Observations.* The postoperative patency of the implants is followed by palpation of the peripheral pulses, appearance of weakness in the hind legs of the animals and aortagrams. When femoral pulsations can be felt, the aortagram shows patency of the prosthesis. Dogs which were sacrificed or died at various intervals postoperatively were subjected to complete autopsy and the prosthesis removed for gross and histological study. The results of the operations are summarized in table 1. Figures IV, V, VI, and VII illustrate the principal microscopic features. Figures VIII, IX, X, and XI illustrate the gross appearance of these tubes.

Routinely, aortagrams are not only helpful to prove the patency, but also to demonstrate any distortion, shrinkage, or aneurysmal dilation of the grafted segment. Examples of these X-rays are presented in figures XII, XIII, XIV, and XV. Comparisons are made between these aortagrams and the gross appearance of the recovered specimens. Distortion of the lumen at

FIGURE IV

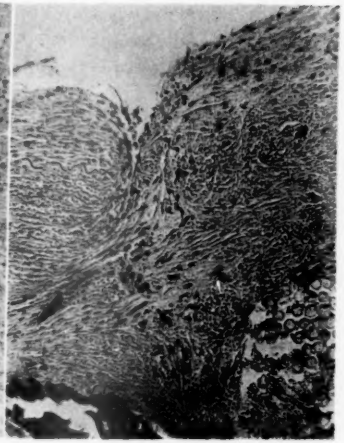


FIGURE V

FIG. IV. Photomicrograph of a longitudinal section through the wall of a fabric prosthesis which was removed four hours after implantation. The interstices of the fabric can be seen to be filled with fibrin and blood cells. A thin mural thrombus lines the luminal surface of the tube, and smears made from this surface showed a marked preponderance of polymorphonuclear leukocytes.

FIG. V. Longitudinal section through the site of anastomosis of a prosthesis which remained in situ for six weeks. The artery on the left may be identified by the elastic and smooth muscle fibres in the media, and the prosthesis, on the right, shows a "sub-intimal" layer which is composed of fibroblasts and collagen fibres lying parallel to the long axis of the prosthesis. A layer of flattened cells covers the entire anastomotic site.

the site of the graft occurred in a few of the earlier operations and consisted of narrowing at the line of anastomosis due to improper suturing, discrepancy in intraluminal size of the graft and the recipient vessel and minor degrees of torsion or kinking incident to excessively long prostheses. Once the technic was standardized and the prostheses accurately tailored to the dimensions of the recipient arteries, these faults disappeared. These observations emphasize the importance of using prostheses tailored to accurate dimensions both as regards length and diameter. In one instance, thrombosis occurred in a distorted prosthesis after having been in place and patent for 30 days. The occlusion occurred a few hours following retrograde aortography and may have resulted from trauma by the tip of the polyethy-

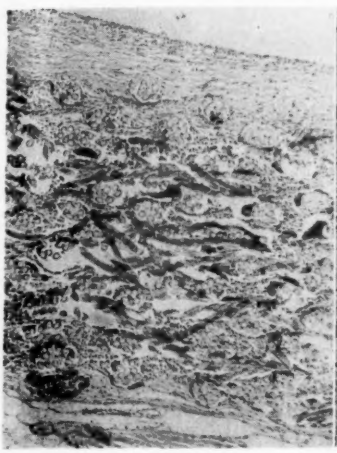


FIGURE VI

FIGURE VII

FIG. VI. Longitudinal section through the wall of a nylon prosthesis removed at 68 days. Invasion of the interstices of the cloth by fibroblasts and capillaries is well illustrated in this photomicrograph.

FIG. VII. Higher power magnification of the section shown in figure VI. Capillaries may be seen to penetrate the interstices of the cloth and to extend into the layer of fibrous tissue lining the inner surface of the tube. The single layer of flattened cells covering the surface of this fibrous tissue may be seen in this photomicrograph.

lene cannula. Another complication of retrograde aortography, using 4 to 6 milliliters of 70 per cent Urokon solution, was the occasional occurrence of paraplegia. The reason for this complication is not known. At autopsy, thrombosis or visible chemical injury to the aorta or to any of the lumbar arteries was not present.

Blakemore and Voorhees (1954) found that separation of the prosthesis from its surrounding bed of connective tissue effectively inhibits the infiltration of the fabric by fibroblasts and capillaries and leads eventually to thrombosis.

Experience gained in the use of these cloth prostheses shows that the following technical points are of importance. 1) Accurate suturing technic is mandatory. 2) The implanted prosthesis must correspond closely to the length and diameter of the excised segment. 3) Meticulous hemostasis must be exercised. Hematomata do not ordinarily result from leakage through the

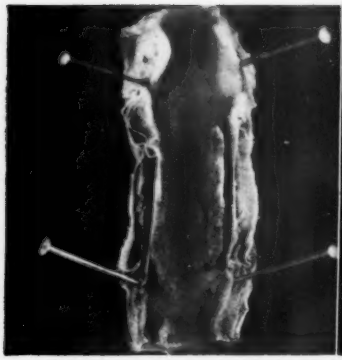


FIGURE VIII

FIGURE IX

FIG. VIII. Gross photograph: Dog No. 3. The fabric prosthesis was removed on the 17th day following implantation. Note the organized mural thrombus which lines the tube. The lumen is fully patent and the thrombus is covered by a smooth layer composed largely of polymorphonuclear leukocytes.

FIG. IX. Gross photograph: Dog No. 15. A prosthesis removed on the 26th postoperative day. Note the smooth, intima-like layer which extends for a distance of several millimeters from either end of the aorta toward the center of the fabric tube.

prosthesis or suture lines but rather from the surrounding tissues. 4) Blood clots and debris must be carefully removed and the field must be perfectly dry before closure. When these precautions are observed and the procedure done in the manner described, a uniformly high percentage of success can be expected.

*Discussion.* Gross and microscopic study of recovered prostheses and surrounding tissues demonstrate the process of repair quite nicely. Initially, there is a layering out of platelets and white blood cells which begins within minutes after blood flow is reestablished through the prosthesis. This pseudo-membrane is essentially a cellular mosaic of polymorphonuclear leukocytes which probably prevents further coagulation of the streaming arterial blood. Very likely, this pseudo-membrane serves to establish the electrotaxic phenomenon observed by Abramson (1927). It is known that the leukocytes and erythrocytes and fibrinogen and other protein elements of the blood normally carry a negative charge. Sawyer and Pate (1953) have demonstrated

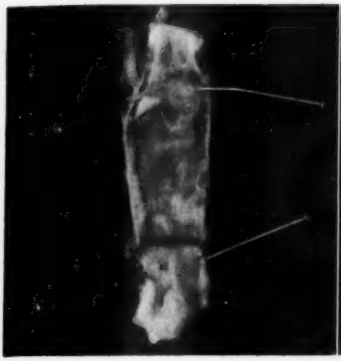


FIGURE X

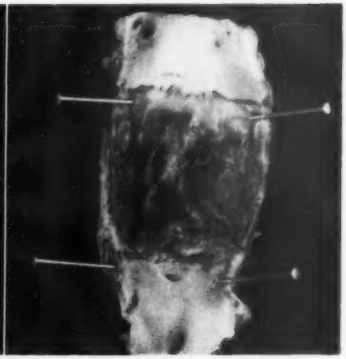


FIGURE XI

FIG. X. Dog No. 5. This prosthesis was removed 45 days following implantation. The glistening fibrous tissue lining completely covers the inner surface of the tube. Two small, partially denuded areas are artefacts produced during the preparation of the specimen.

FIG. XI. Gross photograph: Dog No. 16. This prosthesis was removed 54 days following implantation. The inner surface is elevated and somewhat roughened near the center and represents an incompletely organized mural thrombus. This finding, which was noted in a few of the specimens, probably results from the initial deposition of an unduly thick mural thrombus.

the existence of a difference of electric potential between the intima and adventitia of arteries. Normally, the intima is negatively charged with respect to the adventitia and the sign of this potential difference can be reversed by injury. Intravascular clotting may follow this reversal of potential. These authors have also shown that the freeze-dried arterial homograft has no potential difference after reconstitution by physiologic saline, and this fact, they reason, explains why these grafts do not thrombose. This explanation may apply equally well to implants of inert foreign material. In fact, the prompt filling of the pores of the fabric with fibrin and cellular elements of the circulating blood might be expected to give a small initial negative charge to the inner layer, thereby rendering it isoelectric with respect to the circulating elements of the blood. Later, however, this situation may be reversed by the invasion of fibroblasts from the adventitia surrounding the implant. These relationships might account for the development of the thin mural thrombus attached to the inner surface of the fabric after ten to twenty days. This thin

FIGURE XII

FIGURE XIII

FIG. XII. An aortagram made at 30 days postoperatively shows some dilatation of the prosthesis due to improper selection of a tube of the proper size. This difficulty was eliminated by the practice of accurately tailoring the prosthesis to the recipient artery at the time of operation.

FIG. XIII. An aortagram showing the *in situ* appearance of the prosthesis illustrated in figure IX.

mural thrombus soon becomes organized by fibroblasts and capillary buds invading the interstices of the cloth. The final results is a thin layer of fibrous tissue lining the inside of the fabric tube and covered by flattened cells which are practically indistinguishable from endothelium. This layer is derived, to a considerable extent, by direct ingrowth from each end of the prosthesis. The ingrowth of endothelium extends for a distance of several millimeters from the line of the anastomosis within three or four weeks. Long prostheses develop small glistening islands of endothelium-like tissue on their mid inner surfaces after approximately 45 days. This tissue covers the granulating surface of the organizing thrombus and may be derived from the invading capillary buds.

Depending on the length of the prosthesis, organization of the fabric and establishment of an intact "intima" is usually com-

FIGURE XIV

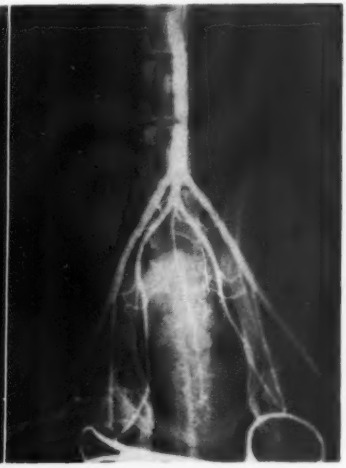


FIGURE XV

FIG. XIV. An aortagram made 30 days postoperatively. The prosthesis is fully patent and there is accurate approximation of the size of the recipient vessel achieved by preparing a prosthesis of the exact size at the time of operation.

FIG. XV. An aortagram made 30 days postoperatively showing patency and accurate approximation of size of the prosthesis. There is, however, a slight defect at the lower end of the prosthesis which was produced by a small mural thrombus.

plete between 60 and 90 days. Although nylon is relatively inert biologically, the fabrics used in this study may have exerted some fibroplastic effect as evidenced by the rather dense, thick layer of fibrous tissue which developed around the prosthesis. The presence of occasional giant cells indicate a mild foreign body reaction to the graft and/or suture material.

While this study is incomplete, it seems evident from the observations made beyond the critical phase of organization of the first three months that these prostheses may be expected to function well indefinitely.

## *Summary and Conclusions*

A method of preparing pervious prostheses from inert fabrics and implanting them to bridge major arterial defects, in-

cluding the bifurcation of the abdominal aorta in dogs, is presented. The results of thirty (30) experiments using this material are presented. Good functional results were obtained in all but one instance. Gross and microscopic study of these prostheses, removed serially, are presented and an effort is made to explain the sequence of events observed following their implantation. The initial findings are sufficiently encouraging to justify further experimental study and clinical application.

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# ELECTRON MICROSCOPY OF ERYTHROCYTES FROM YOUNG CHICKENS EXPERIMENTALLY INFECTED WITH THE LANSING STRAIN OF POLIOMYELITIS*

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Bessis (1950) and Rebuck and Woods (1948) conducted experiments on electron microscopy of erythrocytes and were able to demonstrate structures clearly. Reagan and Brueckner (1953) published electron micrographs of red blood corpuscles from cotton rats bled at intervals following intraperitoneal injection with the Lansing strain of poliomyelitis virus and which showed a few particles similar to poliomyelitis virus.

Until recently it was believed that only primates were susceptible to poliomyelitis, but Armstrong (1939) has shown that the Lansing strain is capable of infecting rodents on intracerebral inoculation.

The poliomyelitis virus used in this study was the mouse-adapted Lansing strain (Armstrong No. 012351) obtained from Dr. Jonas Salk's Laboratory, The Poliomyelitis Research Center, The University of Pittsburgh, and was in the form of a 20 per cent mouse brain suspension. This experiment was undertaken in order to determine if the virus could be demonstrated in association with erythrocytes of chickens inoculated with poliomyelitis.

### *Experimental Methods*

One young chicken (age 6 weeks) was injected in the leg muscle with 2 ml. of a 20 per cent virus-bearing mouse brain suspension of the Lansing strain of poliomyelitis. Another 6-week-old chicken was injected in the leg muscle with a 20 per cent normal mouse brain suspension. Every 24 hours, for 144 hours, the 2 inoculated chickens were bled from the heart and 0.1 ml. of the blood from each chicken was placed into 1 ml. of 0.02 per cent heparin. The six heparinized blood samples from the poliomyelitis injected chicken and the

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six heparinized blood samples from the normal chicken were centrifuged at 2000 r.p.m. for 3 minutes. The supernatents were discarded and the cellular elements were resuspended in 1.0 ml. of sterile distilled $H_2O$. These 12 samples were then placed on parlodion screens which had been prepared the previous week. The screens were then shadowed with chromium (Williams and Wyckoff 1946) at arc tangent 2/12 and examined under the R.C.A. electron microscope, type E.M.U. The balance of the 12 concentrated cellular suspensions was used to intracerebrally inoculate 12 groups of mice in order to determine the infectivity of the samples. Upon symptoms of central nervous system involvement, the mice of the respective groups were sacrificed, their brains were removed, the brains of each group were pooled, ground with alundum and made into a 20 per cent suspension with physiological saline. Neutralization tests with antiserum were then carried out with each group of mouse brain suspensions in order to verify the identity of the virus as being the Lansing strain of poliomyelitis.

## *Results*

Neither the chicken injected with the mouse brain suspension of the Lansing strain of poliomyelitis nor the chicken injected

FIG. 1. Electron micrograph of an erythrocyte from the 24-hour interval bleeding. Virus particles are shown by arrows. Shadowed with chromium at arc tangent 2/12. X 25,000.

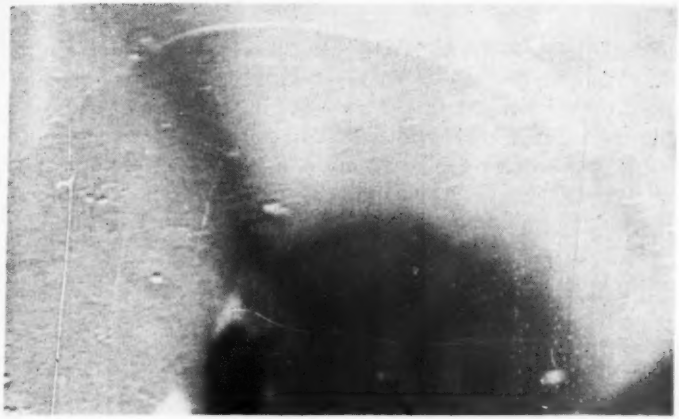

FIG. II. Electron micrograph of an erythrocyte from the 48-hour interval bleeding. Virus particles are shown by arrows. Shadowed with chromium at arc tangent 2/12. X 25,000.

FIG. III. Electron micrograph of an erythrocyte from the 72-hour interval bleeding. No virus-like particles are seen. Shadowed with chromium at arc tangent 2/12. X 25,000.

with the normal mouse brain suspension evidenced any adverse symptoms of central nervous system involvement or malaise. Both were destroyed after the six-day bleeding trial. A study of figures I through VI reveals that virus-like particles are found in the 24-hour, 48-hour, 96-hour, and 120-hour bleedings. Only a few particles were found in the 24-hour and 48-hour bleedings, but a numerous amount were observed in the 96-hour bleeding.

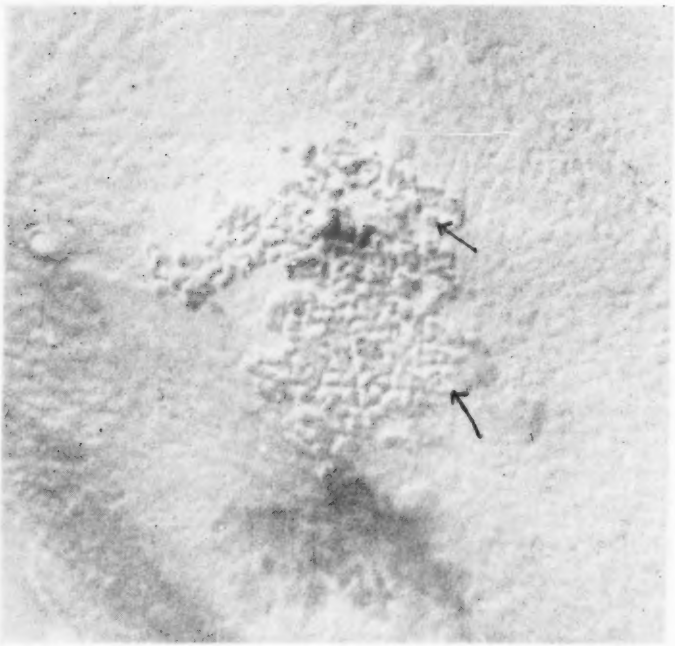

FIG. IV. Electron micrograph of an erythrocyte from the 96-hour interval bleeding. Virus particles are shown by arrows. Shadowed with chromium at arc tangent 2/12. X 25,000.

Figure VII is an electron micrograph of an erythrocyte from the normal chicken and no virus-like particles are evident. Extensive microscopic examination of blood from all 6 bleedings of the normal chicken failed to demonstrate any virus-like particles in any of the erythrocytes.

Table 1 shows the response of the Swiss albino mice injected intracerebrally with the twelve specimens of blood from the infected chicken and the normal chicken. These 12 groups of mice were all held for 21 days. Only the mice inoculated with the 96-hour bleeding specimen evidenced symptoms of central nervous system involvement. This group of mice were sacrificed, their

FIG. V. Electron micrograph of an erythrocyte from the 120-hour interval bleeding. Virus particles are seen. Shadowed with chromium at arc tangent 2/12. X 25,000.

brains were removed, pooled, ground with alundum, and made into a 20 per cent suspension with physiological saline. A neutralization test conducted with appropriate antiserum proved the mouse brain suspension to contain Lansing strain poliomyelitis

TABLE 1

*Response of mice to intracerebral inoculation with concentrated red blood cell suspensions from a chicken injected with a poliomyelitis virus-bearing mouse brain suspension and from a chicken injected with a normal mouse brain suspension.*

| Interval bleeding | Blood from polio chicken | Blood from normal chicken | Incubation period in days |
|---|---|---|---|
| 24 hours | — | — | — |
| 48 hours | — | — | — |
| 72 hours | — | — | — |
| 96 hours | + | — | 8 days |
| 120 hours | — | — | — |
| 144 hours | — | — | — |

FIG. VI. Electron micrograph of an erythrocyte from a 144-hour interval bleeding. No virus particles are seen. Shadowed with chromium at arc tangent 2/12. X 25,000.

virus. The fact that the 96-hour bleeding proved infectious for the mice verifies the finding of numerous virus-like particles in the electron micrograph from this specimen.

*Summary*. One six-week-old chicken was injected in the leg muscle with 2 ml. of a 20 per cent mouse brain suspension of Lansing strain poliomyelitis and another six-week-old chicken was injected in the leg muscle with 2 ml. of normal mouse brain suspension. Neither chicken appeared ill. Each 24 hours, for 144 hours, the two chickens were bled from the heart and 0.1 ml. of each blood specimen from each chicken was placed into 1 ml. of 0.02 per cent heparin. At the end of 6 days a portion of each of

FIG. VII. Electron micrograph of an erythrocyte from the normal chicken. No virus particles are seen. Shadowed with chromium at art tangent 2/12. X 25,000.

the 12 specimens were prepared for electron microscopic studies and the remaining portion was used to inoculate 12 groups of Swiss albino mice intracerebrally. Electron micrographs revealed the presence of a very minute amount of virus-like particles associated with the erythrocyte in the 24-hour, 48-hour, and 120-hour bleeding specimens, and numerous virus-like particles on the erythrocytes from the 96-hour bleeding specimen. No virus-like particles were observed in the 72-hour or 144-hour specimens. Electron micrographs of blood from the normal chicken revealed no virus-like particles. Only mice inoculated with blood from the 96-hour specimen proved to be infectious for the mice which verifies the finding of numerous virus-like particles in this same specimen. A neutralization test with appropriate anti-serum showed the infected mice to contain the Lansing strain of poliomyelitis virus.

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# ELECTRON MICROGRAPHS OF NEGRI BODIES FOUND IN RABIES*

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Specific inclusion bodies present in the central nervous system of man and animals dying of rabies are called "Negri bodies" from their discoverer. These are most frequently demonstrated within, but occasionally outside of, the nerve cells. A definite diagnosis of rabies is made upon demonstration of these bodies in the nerve cells of the brain in stained smears or cut stained tissue sections.

These inclusions are eosinophilic staining bodies, usually spherical but also of other shapes, varying from 1 to 30(3) in size. The inner structure shows basophilic staining granules.

Negri, upon discovery of this body, believed it to be a protozoan. According to one theory the Negri body may be composed of elementary bodies, but there has been no entirely satisfactory evidence to indicate that the body is composed of virus particles.

In a previous study electron micrograph examinations were attempted with histological sections of brain tissue containing Negri bodies, but these proved to be unsatisfactory because the electron beam could not penetrate the specimen section used. Tissue pathology has been studied by Hottle *et al* , using the electron microscope, but they failed to show any virus-like particles. Chemical digestion of the brain cells by trypsin was attempted, but the inclusion bodies seemed to be disintegrated in this process. In a previous paper a technique was described (stated below) which demonstrated Negri bodies containing virus-like particles from two rabies strains. In that experiment the Negri bodies were not metallically shadowed. The idea of possibly removing a Negri body by micro-dissection and observing it with the electron microscope was discarded because the electron beam would kill the Negri body and make it impossible to confirm the work by injection of the body into a susceptible host.

* From the Virus Laboratory, Live Stock Sanitary Service, University of Maryland, College Park, Maryland. Received for publication July 6, 1954.

This experiment was undertaken in order to attempt to confirm the above published work, to use the technique with a different rabies virus strain, and to attempt to bring out more detail by shadowing the Negri bodies with 50 mg. of chromium.

*Methods.* The virus (strain No. 35723) employed in this study was obtained from a dog brain at the Department of Public Health in Baltimore, Maryland. The hippocampus was removed from the brain aseptically and placed in glycerine. Upon receipt at our laboratory the hippocampus was removed from the glycerine, ground with alundum in a mortar, and diluted with physiological saline to a 10 per cent suspension. Three-hundredths ml. of this 10 per cent suspension was injected intracerebrally into each of 10 3-week-old Swiss albino mice (Webster strain). On the 10th day after injection of the strain the mice showed tremors and paralysis and became moribund. The brains were removed aspetically from these moribund mice. Impressions made from the Ammon's horn of each mouse were stained with Sellers stain (6) and under the light microscope showed numerous, large Negri bodies throughout the tissue. The Ammons' horn from two of the mice contained a greater number of Negri bodies than the others and these were used for the experiment below.

Parlodion film supports were prepared 7 days before being used. The prepared screen was picked up with forceps and the parlodion surface was dipped in sterile distilled water. The parlodion surface was then touched lightly to the same area of the Ammon's horn which had been used for the direct smear examination. A portion of this same material was made into a 10 per cent suspension and injected intracerebrally into mice. Six days post inoculation the mice evidenced rabies symptoms. Their brains were removed aseptically, used for touch preparations, stained with Sellers stain, and examined for Negri bodies which were found in abundance. The above prepared screens were alowed to dry, shadowed with chromium (7), and examined under the EMU electron microscope.

Several screens from the strain were examined at various magnifications for inclusion bodies. It was noted throughout this study that definite virus-like particles could be observed in many of the inclusion bodies, but that the internal structure could not be demonstrated in all inclusion bodies; probably due to the combination of cell wall and body thickness, or the latter alone, which blocked electron penetration. The electron beam from a Bias gun was tried but this beam failed to bring out the structure of the Negri body in any more detail, despite the increase in the intensity of electrons.

*Discussion.* Electron micrographs of parlodion - prepared screens of Negri bodies show that it is possible for electrons to penetrate some of the inclusion or Negri bodies. An indefinite internal structure was demonstrated in the strain examined as shown in figures I and II. The Bias gun failed to bring out any more detail than the ordinary EMU electron gun. Figures I and

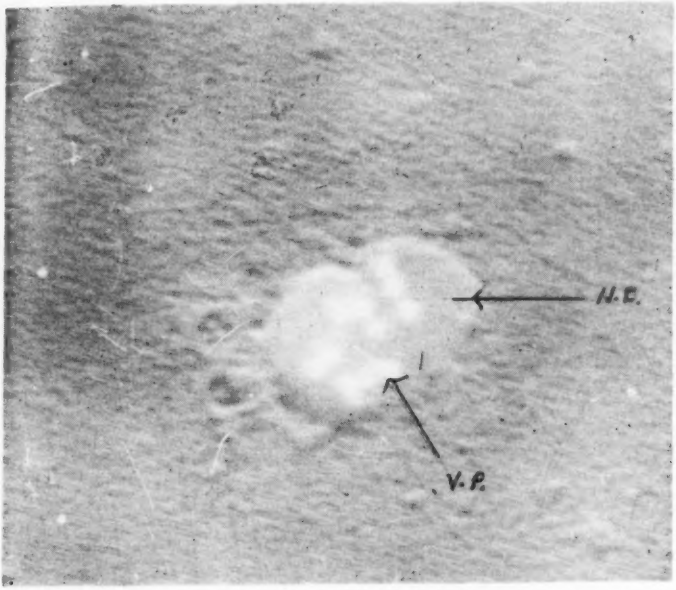


FIG. I. Electron micrographs of a Negri body showing virus-like particles at different magnifications. Arrows indicate Negri body (N.B.) and virus-like particles (V.P.). Shadowed with chromium at arc tangent 2/10. X 15,000 and 10,000.

II show unstained and chromium shadowed preparations containing definite virus-like particles. Figure III shows Negri bodies containing no virus-like particles. The reason why virus-like particles do not show in all of the inclusion bodies can not be explained unless it is due to a variation in the inclusion body thickness.

*Summary*. Negri bodies in this electron microscopic study seem to have definite internal structure which is in the form of elementary bodies. It was not possible to bring out more detail, probably due in some cases to the combined thickness and density of the Negri body, which partly blocks electron penetration.

This technique of specimen preparation for electron studies seems to be satisfactory on examination of this rabies strain and confirms our previous work with two other rabies strains. A

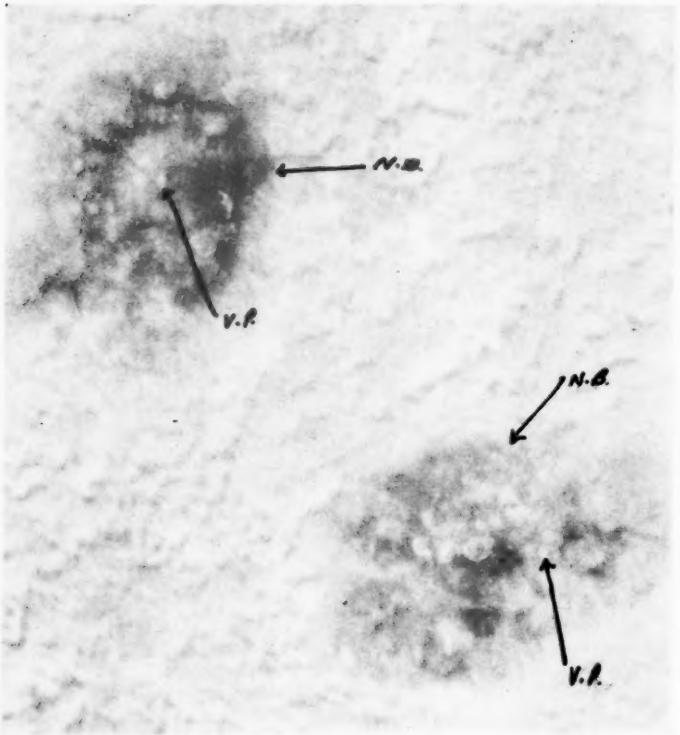


FIG. II. Electron micrographs of two Negri bodies with virus-like particles. Arrows indicate Negri body (N.B.) and virus-like particles (V.P.). Shadowed with chromium at arc tangent 2/10. X 15,000.

disinfectant was not used in order to prevent killing the Negri body area which was used for injection of mice to demonstrate its viability and identity.

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FIG. III. Electron micrographs of a Negri body without virus-like particles. Arrow indicates Negri body. Shadowed with chromium at arc tangent 2/10. X 15,000.

# WHERE CANCER OF THE LUNG OCCURS IN TEXAS*

R. H. Rigdon, Helen Kirchoff, and Norma Martin

Cancer of the lung today is attracting more attention medically, sociologically, and economically than all other neoplasms combined. Cigarette stock fell following a medical symposium, a radio commentator predicted a decrease in the male population fifty years from now and scientists vary in their opinion as to the cause of the increase in the frequency of cancer of the lung.

Cancer of the lung, like all malignant diseases, is occurring more frequently today than ever before. In a group of 29,372 autopsied cases reported between 1937 and 1950 from several different medical centers, cancer of the lung comprised 8.42 per cent of all malignancies (Rigdon and Kirchoff, 1951). Steiner (1953) recently compiled a much larger series of cases of cancer of the lung and noted that in Vienna, between 1915 and 1918, cancer of the lung constituted 10.7 per cent of all autopsied deaths with malignant disease. In a recent survey the United States Public Health Service (1950–52) reported the incidence of cancer of the lung in ten cities in this country. The highest rate was 20.8 cases per 100,000 population in New Orleans and the lowest was 8.9 cases per 100,000 in Atlanta, Georgia. Rigdon and Kirchoff (1952) have shown that the frequency of death from cancer of the lung was related to the ratio of physicians to population and the ratio of hospital beds to population: where there are many doctors and many hospital beds there are more cases of cancer of the lung.

A survey of the data on cancer of the lung in the senatorial districts of Texas was reported in 1950 by Rigdon and Brindley. The distribution of the cases did not suggest that any specific factor played a significant role in the etiology of pulmonary neoplasms. Table 1 gives the total number of deaths from all malignancies, cancer of the respiratory system and cancer of the lung, as reported to the Texas State Department of Health for the years 1948, 1950 and 1952. It is of interest to observe that the

* From the Laboratory of Experimental Pathology, The University of Texas Medical Branch, Galveston. This is part of a cooperative study with the Medical College of Virginia.

TABLE 1

*Reported Cancer and Cancer of the Lung Deaths in Texas 1948, 1950 and 1952*

| | 1948 | 1950 | 1952 |
|---|---|---|---|
| All malignancies ..... | 6,899 | 8,095 | 8,701 |
| Malignancies of the respiratory tract ..... | 674 | 832 | 1,038 |
| Carcinoma of the lung..... | 477 | 700 | 911 |
| Malignancies of the respiratory tract other than lung ..... | 197 | 132 | 127 |

Data obtained from Texas State Dept. of Health, 1954.

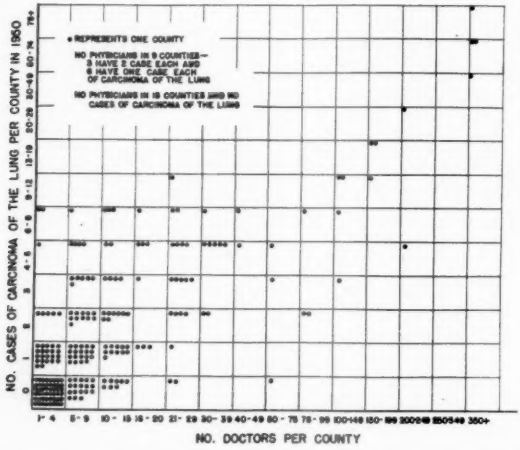
number of reported deaths from cancer of the lung almost doubled (477 to 911) in the period from 1948 to 1952. During this same time a decrease (197 to 127) occurred in the number of deaths from neoplasms in the respiratory system other than of the lung.

To see where cancer of the lung was occurring in the State of Texas data were obtained for each county. Combining the reported cases of cancer of the lung for 1948, 1950, and 1952, it was found that thirty-nine counties had reported no deaths and thirty-nine other counties had each reported only one death from cancer of the lung during these three years. These counties without any reported deaths from cancer of the lung were distributed throughout the state. Because of this distribution, no significance could be attributed to local environmental factors in the etiology of this neoplasm. It has been pointed out by Doll and Hill (1953) and Kennaway and Waller (1953) that cancer of the lung occurs more frequently in city and urban populations than in rural peoples.

In view of our previous study on the relation of pulmonary cancer to the number of physicians in the different states (Rigdon and Kirchoff 1952) a similar study was made referable to the number of reported deaths from cancer of the lung in 1950 and the number of physicians in the different counties of Texas. Graph I shows the relation of cancer of the lung to the number of physicians in each county.[1] It is obvious from these data that more deaths from cancer of the lung are reported from counties with a large number of doctors.

[1] Data on number of physicians obtained from Texas Medical Association and represents only physicians now members of this association.

Usually it is true that a county with a large population will have more doctors than one with fewer residents. The physician to person ratio was determined for each county and this was correlated with the number of reported deaths from cancer of the lung in 1950. These data show the wide variation in the number of doctors in relation to the population in the different counties. The extremes are one doctor for every 438 persons and one for


• REPRESENTS ONE COUNTY
NO PHYSICIANS IN 9 COUNTIES—
9 HAVE 2 CASE EACH AND
6 HAVE ONE CASE EACH
OF CARCINOMA OF THE LUNG
NO PHYSICIANS IN 15 COUNTIES AND NO
CASES OF CARCINOMA OF THE LUNG
NO. CASES OF CARCINOMA OF THE LUNG PER COUNTY IN 1950
NO. DOCTORS PER COUNTY

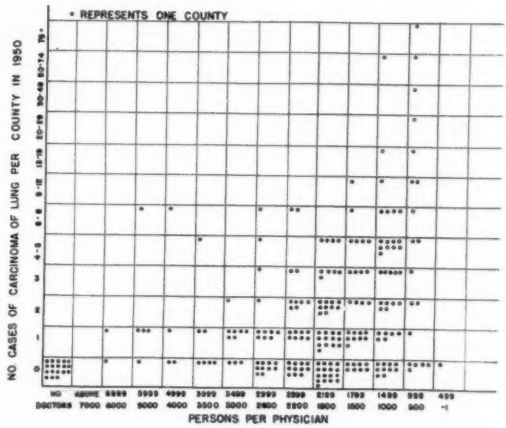
GRAPH I. Shows the relation of cancer of the lung to the number of physicians in each county in Texas for 1950.

8.751. Graph II indicates that the number of reported deaths from cancer of the lung in the different counties is related to the ratio of physician to population: where there are many doctors there are more deaths reported from cancer of the lung.

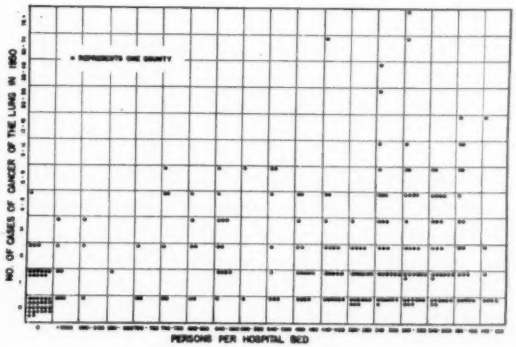
The relation of the frequency of cancer of the lung to the number of general hospital beds[2] in 1950 was studied. The ratio of beds to population in each county was determined. This was plotted against the number of cases of cancer of the lung reported from each county (Graph III). These data show that the number of reported cases of cancer of the lung increases with the increase in the ratio of general hospital beds to population.

[2] Texas State Plan for Construction of Hospitals and Public Health Centers. Revised July 1, 1951. Published by Texas State Department of Health.

The above observations support the opinion that the increase in frequency of cancer of the lung is related to better diagnosis. Steiner (1953) has shown that the increase in frequency of cancer of the lung is related to the introduction of newer methods for the clinical diagnosis of this type of neoplasm. Time and ac-




GRAPH II. Indicates that the number of reported deaths from cancer of the lung in the different counties in 1950 is related to the ratio of physician to population.

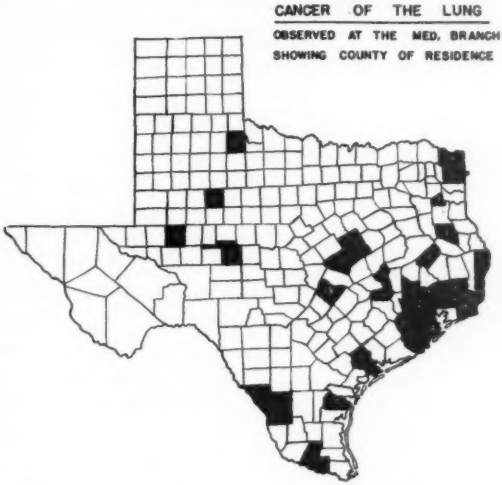



GRAPH III. Gives the number of reported cases of cancer of the lung and the number of hospital beds per person for each county in Texas for 1950.

cessory equipment are needed for the diagnosis of this malignancy. Even with these assets the diagnosis of primary carcinoma of the lung frequently is not easy. Recently we had an opportunity to make a postmortem examination on a white male 39 years of age who had been thoroughly examined in the clinic of an internationally known surgeon only three months preceding death, at which time no neoplasm was found. At autopsy, there was a squamous cell carcinoma involving the left upper lobe of the lung with extensive metastases to the lumbar vertebrae and viscera. Every physician has had such an experience with this type of neoplasm. Even pathologists have been known to disagree on the diagnosis of cancer of the lung. They, of all people, are conscious of the difficulties in the clinical diagnosis of primary carcinoma of the lung. Such problems must be carefully evaluated in any statistical study of the frequency of cancer of the lung.

Thoracic surgeons and medical centers have been emphasizing the increase in the frequency of cancer of the lung during the past ten years. A review of the number of cases of cancer of the lung observed by the physicians in the majority of the counties in Texas does not suggest that they are seeing a significant increase in this type of malignancy. To study this problem, fifty cases of cancer of the lung recently seen at the Medical Branch Hospitals in Galveston were reviewed to determine the county of residence. Patients from the entire State of Texas are referred to this institution. Graph IV shows the county of residence of these fifty cases. The largest number of patients with cancer of the lung seen at the Medical Branch Hospitals were not residents of Galveston County. Apparently a similar situation occurs in other centers where diagnostic facilities are available. It becomes obvious that diagnostic facilities, the number of physicians and the number of hospital beds are very important in the overall study of the increase in the frequency of cancer of the lung.

The factors cited in this paper appear to us to be very important when the etiology of pulmonary cancer is considered. When only our hospital records are considered, Galveston County might be considered statistically a focus within the state in which cancer of the lung is increasing. Cancer of the lung is increasing in Los Angeles and the smog is being considered as a factor. Cancer of the lung is increasing in Texas and there is no smog.




GRAPH IV. Shows the county of residence of 50 patients with cancer of the lung seen at the Medical Branch Hospitals, Galveston.

Hueper (1952) said that "the geographical distribution pattern displayed by respiratory cancers clearly suggests the action of an environmental agent which is present or operative to a higher degree in urban and industrialized regions than in rural areas. Air pollution from effluents of domestic fireplaces, incinerators, industrial establishments, and carbon black plants, exhaust fumes from gasoline and diesel engines and coal or oil fired railroad locomotives, dust from asphalted, tarred and oiled roads and from abrasion of rubber tires (Sharrad) would perhaps best conform with this pattern." The most frequent agent being considered today as the etiologic agent for cancer of the lung is the cigarette.

The "lag-period" becomes very important in considering the relation of any etiologic agent to cancer of the lung. Wynder and associates (1953) have suggested that such a period is approximately thirty-five years in the case of cigarettes. Hueper (1954) states that various investigators have estimated this latent period to be from twenty to forty years. The annual consumption of cigarettes has increased rapidly and in essentially a straight line since 1915 (Hueper, 1954). In Texas the number of reported deaths from carcinoma of the lung was 477 in 1948 and 911 in

1952. There is no available data on the consumption of cigarettes in Texas from 1915 to 1930. However, there are no variations observed in the annual consumption of cigarettes in the United States that would account for this rapid rise at this particular time as far as we know. Furthermore, Texas was an agricultural state with few industries before 1940. There is reason to believe that the consumption of cigarettes was less in Texas between 1920 and 1930 than in industrial states. Doll in 1953 pointed out that there is much less cigarette smoking in rural areas of England than there is in cities. In considering any etiologic agent as the cause of lung cancer, we must consider the environmental status of the population twenty to forty years ago, if we accept the idea that such a latent period does occur.

*Summary*. The frequency of cancer of the lung has been reviewed in the 254 counties in Texas. These data have been correlated with (a) the population as recorded in the census of 1950, (b) the number of general hospital beds in 1951, and (c) the number of physicians now members of the State Medical Association. From these data it may be concluded that the number of deaths from cancer of the lung in each county is related to the number of doctors and the number of hospital beds in the county. Better diagnosis, therefore, is a significant factor in the increase in the frequency of cancer of the lung. Factors of better diagnosis and the distribution of cases of cancer of the lung through the state must be carefully evaluated when any specific etiologic agent is proposed as the cause of cancer of the lung.

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# A CONSIDERATION OF THE MECHANISM OF RETICULOCYTOSIS: AN EXPERIMENTAL STUDY IN THE DUCK*

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The duck is an excellent host for an experimental study on erythropoiesis because of its rapidity of growth and the large number of reticulocytes normally present in the peripheral blood of young birds. Many of the normal hematologic values now have been established for the white Pekin duck (Hewitt, 1942; Rostorfer, 1946; Crass, 1954; Magath, 1934). Reticulocytes in the peripheral blood rapidly decrease when selenium is given (Rigdon, 1953). This diminution has been attributed to an inhibition in the process of maturation. Cysteine inhibits the effect of selenium on the bone marrow (Rigdon, 1953). There is a rapid increase in the number of reticulocytes when phenylhydrazine is given orally (Rigdon, 1953). Chloramphenicol produces a rapid diminution in the number of reticulocytes in the peripheral blood (Rigdon, 1954). This is accompanied by degenerative changes in the cells within the bone marrow. Malaria-infected ducks show a rapid increase in the number of reticulocytes in the peripheral blood (Rigdon, 1946). Cobalt, as one might expect, results in a polycythemia (Davis, 1945). Starvation causes a complete disappearance of reticulocytes from the peripheral blood within less than 72 hours; the reticulocytes return within a similar interval when food is given fasting ducks (Rigdon, 1954).

This variety of responses in the reticulocytes of the duck has stimulated us to investigate the mechanism of reticulocytosis. In this study two basic problems have presented themselves—nutrition and anoxia.

*Methods and Material.* White Pekin ducks, obtained from a commercial hatchery when one day of age, were kept in small batteries in the laboratory. The primary ration was duck Star-

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tena and Growena prepared by Ralston Purina Company of St. Louis, Mo. Food and water were available at all times. The age of the birds, their specific food and the details of each experiment are given elsewhere in this paper.

Blood for the erythrocyte and reticulocyte counts was obtained from the web of the foot. Standard techniques were used for the red cell counts. A one per cent aqueous solution of crystal violet was used for staining the reticulocytes. The percentage of reticulocytes was determined by counting 1,000 erythrocytes. This technique has been used in several previous studies published from this laboratory (Rigdon, 1953, 1954). The technique for the bone marrow examination has been described elsewhere (Crass, 1954). Absolute attention to technique in obtaining, fixing and staining is essential for good differentiation of bone marrow cells in the duck.

*Effect of Starvation on the Number of Reticulocytes in the Peripheral Blood.* In this experiment four ducks 22 days of age were used. After the normal number of erythrocytes and reticulocytes was established, all food was removed from two birds for six days and two ducks were kept for the controls. The results of this experiment are shown in figure I. The reticulocytes in the fasting birds completely disappeared from the peripheral blood within 72 hours. The reticulocytes returned within a similar period when food was replaced. It is interesting to note the large number of reticulocytes that appeared for a short interval after the food was replaced. Such "over compensation" has been observed frequently in other experiments (Rigdon, 1953).

Few observations have been made to determine the effect of a reduction in the food intake on the number of reticulocytes in the peripheral blood. To study this four ducks approximately the same size and age as those used in the above experiment were given 35 grams of the Purina Growena food per day. Normally ducks of this size consume approximately 90 grams per day. The number of reticulocytes rapidly decreased in the peripheral blood at a rate essentially the same as that observed when all food was withdrawn. In another experiment (Fig. II) four ducks 19 days of age, were given 60 grams of food each day for four days. There was a reduction in the number of reticulocytes; however, the decrease was slower than it was when only 35 grams

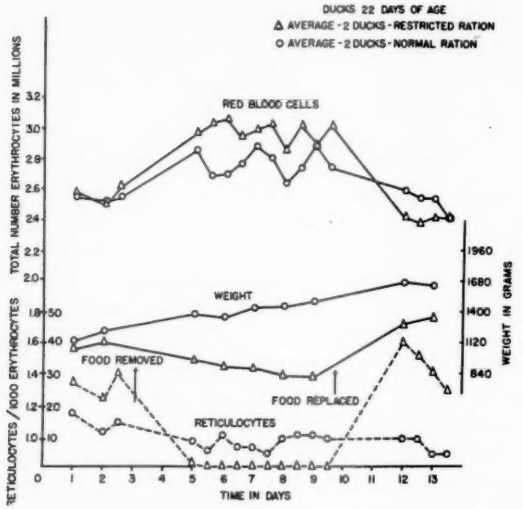


FIG. I. Reticulocytes rapidly decrease in the peripheral blood when food is discontinued and they rapidly return when food is replaced.

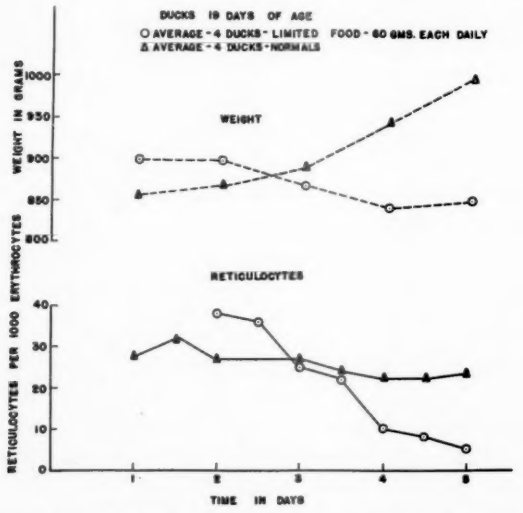
DUCKS 19 DAYS OF AGE
○ AVERAGE - 4 DUCKS - LIMITED FOOD - 60 GMS. EACH DAILY
Δ AVERAGE - 4 DUCKS - NORMALS
WEIGHT IN GRAMS
WEIGHT
RETICULOCYTES
RETICULOCYTES PER 1000 ERYTHROCYTES
TIME IN DAYS

FIG. II. With a reduction in the food intake there occurred a proportional decrease in the number of reticulocytes in the peripheral blood.

of food were given. Reticulocytes persisted in the peripheral blood during the time of the experiment as shown in figure II.

The percentage of myeloid and erythroid cells in the bone marrow of nineteen ducks, eight of which received no food and four a slight reduction in their food intake over a period of four days, is shown in table 1. The percentage of erythroid cells de-

TABLE 1

*Number of Cells in Bone Marrow During Starvation*

| Ducks | Age in Days | Amount food in gms | Cells in Bone Marrow | |
|---|---|---|---|---|
| | | | Erythroid | Myeloid |
| 637 | 18 | Ad. Lib. | 42.0 | 58.0 |
| 638 | 18 | Ad. Lib. | 41.6 | 58.4 |
| 639 | 18 | Ad. Lib. | 39.8 | 60.2 |
| 640 | 18 | Ad. Lib. | 42.9 | 57.1 |
| Average | | | 41.6 | 58.4 |
| 629 | 18 | None | 24.6 | 75.4 |
| 630 | 18 | None | 24.0 | 76.0 |
| 631 | 18 | None | 27.1 | 72.9 |
| 632 | 18 | None | 20.7 | 79.3 |
| 633 | 18 | None | 21.1 | 78.9 |
| 634 | 18 | None | 20.2 | 79.8 |
| 635 | 18 | None | 24.8 | 75.2 |
| 636 | 18 | None | 22.1 | 77.9 |
| Average | | | 23.1 | 76.8 |
| 621 | 23 | Ad. Lib. | 39.3 | 60.7 |
| 622 | 23 | Ad. Lib. | 41.0 | 59.0 |
| 623 | 23 | Ad. Lib. | 38.9 | 61.1 |
| Average | | | 39.7 | 60.3 |
| 625 | 23 | 60 | 29.4 | 70.6 |
| 626 | 23 | 60 | 33.1 | 66.9 |
| 627 | 23 | 60 | 29.1 | 70.9 |
| 628 | 23 | 60 | 32.6 | 67.4 |
| Average | | | 31.5 | 68.9 |

creased, while that of the myeloid cells increased when all food was withdrawn. When the amount of food intake was reduced approximately two-thirds there was a corresponding decrease in the proportion of erythroid cells within the bone marrow as shown in table 1. These observations on the bone marrow would

indicate that the variations occurring in the reticulocytes in the peripheral blood are a true representation of the changes occurring within the bone marrow.

From these studies it is obvious that something is present in the Purina ration that influences the formation of reticulocytes. Water was restricted but this had no significant effect on the reticulocytes as long as this food was given.

*Effect of Certain of the Vitamins on Reticulocytosis.* Two vitamins, $B_{12}$[1] and folic acid, known to play significant roles in hematopoiesis in mammals, were selected to study their effect on the formation of reticulocytes in the duck. Two birds, 14 days of age were fed *ad libitum*, and two ducks of similar age were given no food and 200 mcg. of vitamin $B_{12}$ subcutaneously three times daily for four days. There were no reticulocytes in the peripheral blood of the latter two ducks after 72 hours. The two control birds had an average of 40 reticulocytes per 1,000 red blood cells in the peripheral blood after four days.

Two ducks, 31 days of age had their food discontinued and were given 50 mg. of folic acid orally twice daily for 4 days. The reticulocytes rapidly decreased and none were demonstrated in the peripheral blood after 72 hours. Two ducks of similar age were given the same amount of folic acid and also 200 mcg. of vitamin $B_{12}$ were given subcutaneously three times per day for 4 days. The reticulocytes were completely absent from the peripheral blood of these latter two birds within a period of 72 hours.

In view of these experiments with vitamin $B_{12}$ and folic acid it would appear that neither of these two vitamins are primarily related to this process of rapid diminution of reticulocytes in the peripheral blood when food is withdrawn from ducks.

*Effect of Certain Minerals on Erythropoiesis.* Cobaltous chloride and iron were studied to determine their effect on the formation of reticulocytes in the duck. Four ducks 18 days of age were fed the Purina ration and were given 500 mg. of cobalt chloride orally once each day on the 2, 3, 4, 5, 6, 8, 9, 10, and 11th day of the experiment. These four ducks progressively gained in weight and the number of reticulocytes slowly increased in num-

[1] The vitamin $B_{12}$ and the Amino Acids used in this study were supplied by Merck & Co. through the kindness of A. W. Veazey.

ber in the peripheral blood. After 12 days the average number of reticulocytes in the peripheral blood of these four birds was 42 per 1,000 red blood cells. The average number of reticulocytes in the peripheral blood of four ducks of the same age, fed only the Purina ration during this interval, was 30 per 1,000 erythrocytes. There were no reticulocytes within the peripheral blood, after 72 hours, in two ducks of equal age given the same amount of cobalt and no food for four days.

From this experiment it is evident that a slight rise occurs in the number of reticulocytes within the peripheral blood when large amounts of cobaltous chloride are given to ducks receiving an adequate food intake; however, the reticulocytes rapidly decrease in the peripheral blood of ducks given large amounts of cobalt with no food intake.

Two ducks 19 days of age had their food discontinued and were given orally 0.3 gms. ferrous sulfate per day for five days. The number of reticulocytes rapidly decreased and within 72 hours all these cells were absent from the peripheral blood. Two ducks of similar age had their food discontinued and on the third day there were no reticulocytes in the peripheral blood. At this time they were given 0.3 gms. of ferrous sulfate daily for two days. The reticulocytes remained absent from the peripheral blood during the next two days. These observations would suggest that the absence of iron is not the primary factor responsible for the rapid decrease in reticulocytes in the peripheral blood when all food is discontinued.

*Effect of Carbohydrates and Fats on the Formation of Reticulocytes.* Eight ducks 21 days of age were used in this experiment. All food was discontinued. Two birds were given sucrose (cane sugar), two fat (Wesson oil), two celluluose (Alphacel[2]) and two were kept as the controls. The sucrose and vegetable oil were given on the basis of approximately 500 calories per duck per day. This amounted to 127 grams of sucrose (42 gms. t.i.d.) and 54 gms. of Wesson oil (18 ml. t.i.d.). Sixty milliliters (dry) of cellulose were given two ducks (20 ml. t.i.d.). All ducks were force-fed during this experiment and were given water *ad libitum*. The variation in the number of reticulocytes in the peripheral blood and the weight curves on these 8 birds are shown in

[2] Prepared by Nutritional Biochemical Corporation, Cleveland, Ohio.

figure III. A rapid diminution occurred in the number of reticulocytes in the sucrose, fat and cellulose fed birds, similar to that of the two fasting birds. All birds lost weight at approximately the same rate. Probably the weight curve remained slightly higher in the two ducks receiving the Wesson oil than it did in the other six birds.

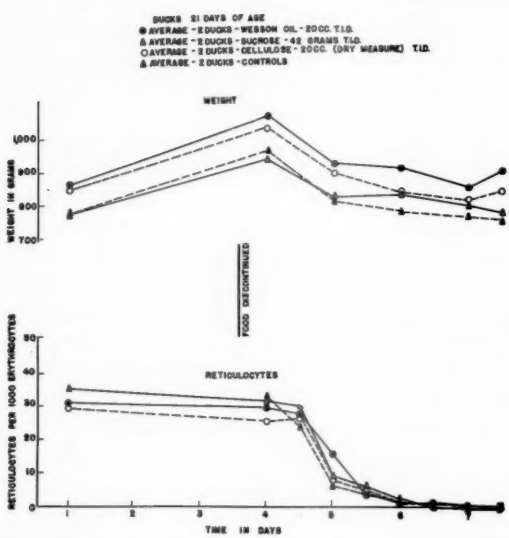


FIG. III. Reticulocytes rapidly disappeared from the peripheral blood of all birds in this experiment. The rate of disappearance was the same in those receiving sucrose and Wesson oil as in those without food.

Obviously carbohydrate (sucrose) and fat (Wesson oil) are not the significant factors in duck rations necessary for the formation of reticulocytes as shown by these experiments.

*Effect of Specific Proteins and Amino Acids on Reticulocyte Formation in the Duck.* The protein used in this experiment was casein[3] (vitamin free). The amino acids were a group of eleven supplied by Merck & Co. containing the following:

[3] Prepared by General Biochemicals Inc., Cleveland, Ohio.

| | Grams per liter |
|---|---|
| L-Arginine | 2.5 |
| Glycine | 23.0 |
| L-Histidine | 4.2 |
| L-Isoleucine | 8.0 |
| L-Leucine | 16.5 |
| L-Lysine | 9.4 |
| L-Methionine | 4.0 |
| L-Phenylalanine | 5.3 |
| L-Threonine | 1.9 |
| DL-Tryptophan | 1.8 |
| L-Valine | 7.0 |
| L-Glutamic Acid | Max. 1.5 |
| Lactic Acid | 10.0 |

A commercial high protein supplement[4] was given to two ducks. These three preparations (a) casein, (b) amino acids, and (c) high protein supplement were fed either as a water solution or suspension so that each duck received 45 grams per day. The birds were force-fed several times each day. The number of reticulocytes and the weight curves on these three groups of birds are shown in figure IV. The reticulocytes in the two birds without food rapidly decreased, a similar decrease occurred in the two birds given the high protein supplement. The two ducks receiving the combination of amino acids showed a slightly less reduction in reticulocytes than that occurring in the controls. The two ducks given the casein showed a decrease in the number of reticulocytes but the decrease was much less than that in the controls that received no food. The weight loss of the two ducks given the casein, the two receiving the protein supplement and the two given the amino acids was less than that in the two controls as shown in figure IV.

Two ducks 28 days of age were fed a combination of casein and sucrose (45 grams of casein and 79 grams of sucrose, a total of 500 calories per day). Two ducks were used as controls. The weight and the reticulocyte counts are shown in figure V. Note that the two birds receiving the casein and sucrose gained in weight while the controls lost. The reticulocytes in the two controls rapidly decreased while the reticulocytes remained high in

[4] High protein supplement—Smith-Dorsey, Lincoln, Nebraska. Approximate analysis: protein, 60 per cent; fat, 1.5 per cent; carbohydrate, 27 per cent; minerals, 6.3 per cent; moisture, 5.0 per cent; caloric equivalent per gram, 3.6.

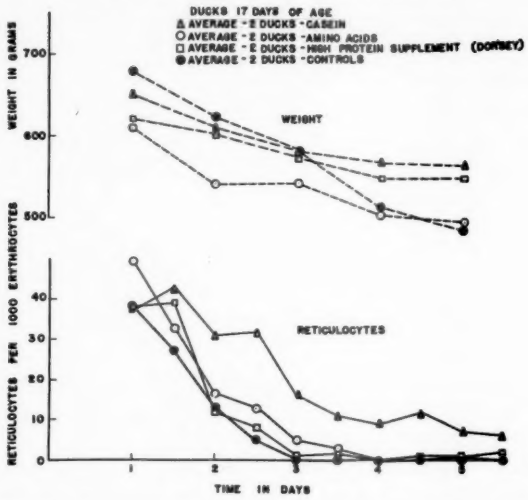


FIG. IV. The decrease in the number of reticulocytes in the peripheral blood was essentially the same when this combination of amino acids and the high protein supplement were fed as that observed when no food was given. When casein was fed the reticulocytes persisted in the peripheral blood.

the two birds receiving the casein and sucrose—in fact after five days the reticulocyte count in the latter pair of ducks was 75 per cent of the initial count. Normally we expect to have some decrease in the reticulocyte count as the result of aging during this interval. This experiment was repeated on two ducks with similar results.

From this experiment it is obvious that casein is an important nutritional substance in preventing the rapid decrease in the reticulocytes when all other foods are withdrawn. Furthermore, casein and sucrose together are much better than casein alone in preventing this rapid diminution in the number of reticulocytes. Sucrose alone has no inhibiting effect on the diminution of reticulocytes in the peripheral blood when all other foods are discontinued as shown in figure III.

*Observations on the Reticulocyte Response of Ducks Without Food and Given Phenylhydrazine Hydrochloride.* To study the reticulocyte response of ducks to phenylhydrazine two ducks 38 days of age were fed Purina rations and given orally 65 mgs. of phenylhydrazine hydrochloride in 3.5 ml. of 95 per cent ethyl

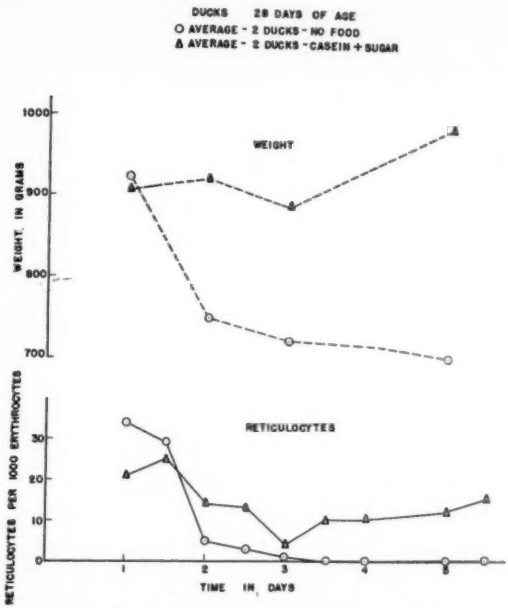


FIG. V. Ducks fed casein and sugar continued to gain in weight and reticulocytes remained in the peripheral blood.

alcohol. Two ducks were given no food and an equal quantity of phenylhydrazine, two ducks were given no food and two other birds were fed the Purina ration. All ducks were followed for a period of twelve days. The results of this experiment are shown in figure VI. The number of reticulocytes in the peripheral blood is approximately the same for the two ducks receiving no food and given the phenylhydrazine as it is for the two ducks receiving food and phenylhydrazine. The peak of the reticulocyte count occurred on the fifth day and within three days the number of reticulocytes had returned to within the range of normal (Fig. VI).

It is of interest to observe this marked reticulocyte response in the ducks not receiving any food and given phenylhydrazine, but it should be remembered that these two ducks had been fed *ad libitum* until the time they were given the phenylhydrazine hydrochloride, a factor that might influence their response.

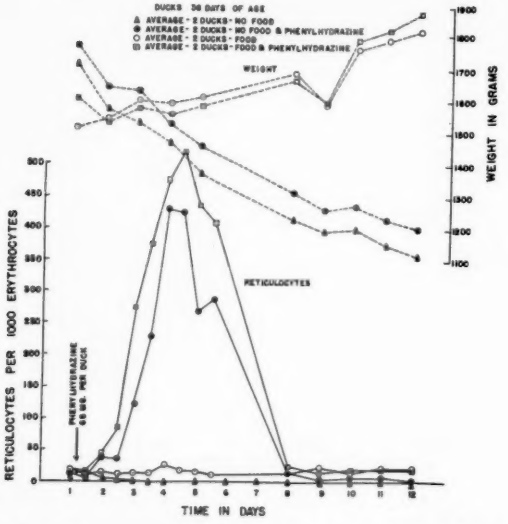
DUCKS 36 DAYS OF AGE
AVERAGE - 2 DUCKS - NO FOOD
AVERAGE - 2 DUCKS - NO FOOD & PHENYLHYDRAZINE
AVERAGE - 2 DUCKS - FOOD
AVERAGE - 2 DUCKS - FOOD & PHENYLHYDRAZINE
WEIGHT
WEIGHT IN GRAMS
RETICULOCYTES
RETICULOCYTES PER 1000 ERYTHROCYTES
PHENYLHYDRAZINE 16 MG. PER DUCK
TIME IN DAYS

FIG. VI. Reticulocytes remained in the peripheral blood of the two normal ducks. These cells disappeared from the blood of the two birds without food. Both groups of ducks given phenylhydrazine showed essentially the same reticulocyte response.

A second experiment was performed in which all food was withdrawn for 72 hours from two ducks (D902 and D903) 32 days of age before they were given a small dose (16 mg.) of phenylhydrazine; the controls (D904 and D905) were fed and given the same amount of phenylhydrazine. The results of this experiment are shown in figure VII. There was essentially no difference in the degree of anemia and reticulocyte response in the two ducks (D902 and D903) that received no food and were given the phenylhydrazine (Fig. VII). The total number of red blood cells in these two ducks rapidly decreased beginning very shortly after the phenylhydrazine was given. The reticulocytes, however, did not appear in the peripheral blood of ducks 902 and 903 until 48 hours later; even then they were few and the total did not approach the number in the peripheral blood observed in duck 905 that was fed during the time of this experiment. The total number of erythrocytes rapidly increased in duck 905 following the administration of the phenylhydrazine.

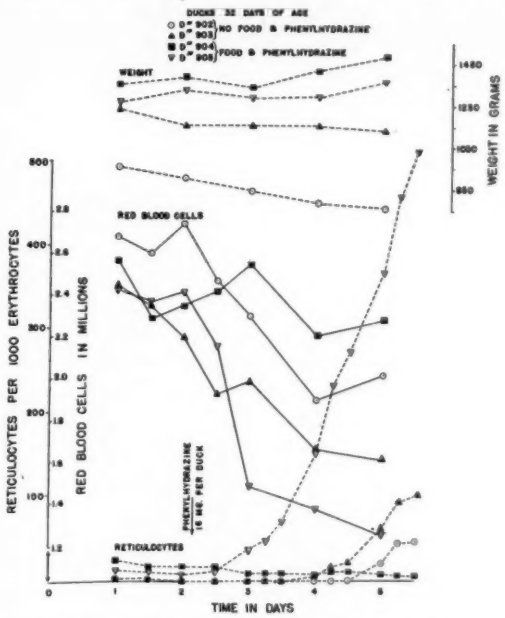


FIG. VII. Two ducks 902 and 903 had received no food for 48 hours and the reticulocytes were absent in the peripheral blood. They were given phenylhydrazine and no food. Note the delay and the poor reticulocyte response. Duck 905 shows a quick and rapid reticulocyte response. Duck 904 shows no response to this minimal dose of phenylhydrazine.

The number of reticulocytes began to increase within 24 hours after the phenylhydrazine was given and continued to increase rapidly during the following 48 hours, at which time the experiment was discontinued. Duck 904 treated the same as duck 905 showed essentially no change in the erythrocyte and reticulocyte counts in response to the phenylhydrazine. Obviously this dose of phenylhydrazine was too small to produce even a minimal change in the erythrocyte and reticulocyte counts in this bird.

The data shown in figure VII indicate that phenylhydrazine in a sufficient amount produces anemia in the white Pekin duck. The reticulocyte response to this anemia is much greater in ducks receiving an adequate ration, as illustrated by duck 905,

than it is in ducks receiving no food, as shown by ducks 902 and 903. The increase in the number of reticulocytes in the peripheral blood also begins to appear much earlier in birds receiving an adequate ration than in birds receiving no food as shown in figure VII.

This experiment with phenylhyrazine would suggest that the reticulocyte response is influenced by two factors, nutrition and anemia. A duck not receiving food and made anemic by phenylhydrazine is unable to produce reticulocytes at the same rate and to the same degree as a duck given phenylhydrazine and an adequate ration *ad libitum*. Furthermore, reticulocytes appear in the peripheral blood in ducks adequately fed and given phenylhydrazine within 24 hours, while ducks without food show only a poor reticulocyte response even after 72 hours as shown in figure VII.

*Reticulocyte Response in Ducks Made Anemic by Bleeding.* In the preceding experiments, when the ducks were made anemic with phenylhydrazine, it was obvious that both food and anoxia were significant factors in the reticulocyte response. Since the anemia produced by this chemical (phenylhydrazine) continued to develop for 48 to 72 hours, an acute and immediate anemia was produced by the sudden withdrawal of 25 ml. of blood from the heart and by the replacement of 20 ml. of duck serum intravenously within an interval of one hour. Two ducks had their food discontinued for 72 hours before the blood was removed. There were no reticulocytes in the peripheral blood of these two ducks at the time they were bled. Two ducks of equal size and age were similarly bled. These birds had 24 reticulocytes per 1,000 erythrocytes in the peripheral blood as shown in figure VIII immediately before the bleeding. The reticulocyte response was much greater and it occurred much sooner in the two ducks receiving food. It would appear therefore that the process of formation of reticulocytes is more efficient in the presence of adequate nutrition. Figure VIII also shows that reticulocytes begin to increase in the peripheral blood much sooner in the birds receiving an adequate ration than in ducks given no food during the time of the experiment.

*Discussion.* Many factors are known to affect the reticulocyte count in both man and animals; however, the rapidity of this

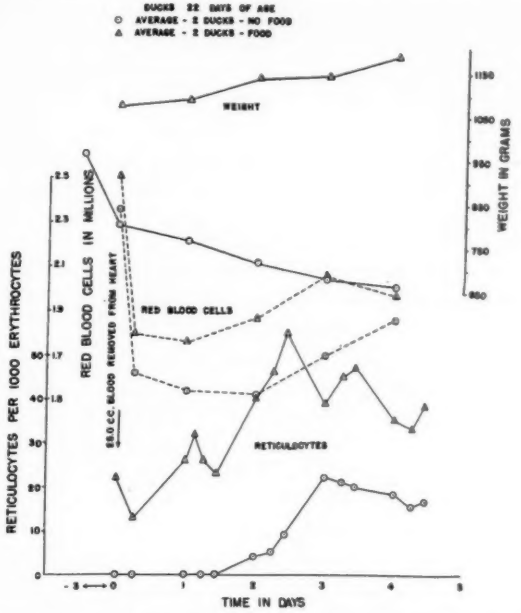
DUCKS 22 DAYS OF AGE
AVERAGE - 2 DUCKS - NO FOOD
AVERAGE - 2 DUCKS - FOOD
WEIGHT
WEIGHT IN GRAMS
RED BLOOD CELLS
RETICULOCYTES
RETICULOCYTES PER 1000 ERYTHROCYTES
RED BLOOD CELLS IN MILLIONS
50 CC. BLOOD REMOVED FROM HEART
TIME IN DAYS

FIG. VIII. Food discontinued in 2 ducks for three days before and five days after bleeding. Note the decrease in the reticulocyte response in these birds when compared with ducks that were fed.

response is much greater in the duck than in mammals. Anemia is universally regarded as a stimulant to reticulocytosis (Wintrobe, 1952). Hypoxia and respiratory insufficiency also cause reticulocytosis in the absence of anemia (Riska, 1950). Obviously we may have the manifestations of anoxia without necessarily having a diminution in the number of erythrocytes, as shown in the polycythemia observed at high altitudes.

In the duck, as well as in many animals, a polycythemia accompanies the administration of cobaltous chloride (Davis, 1945). The mechanism for this increase in erythrocytes has been dicussed frequently (Barron, 1936; Davis, 1940). It has been suggested by Barron and Barron (1936) and Davis (1940) that cobalt may act upon the hemopoietic system by impairing cellular oxidation. Orten and Bucciero (1948) were able to inhibit the production of polycythemia by cobalt in rats by giving

cysteine. It was suggested that "cysteine inhibits the production of polycythemia by cobalt by the formation *in vivo* of an inert cobalt-cysteine complex. . . . The suggestion is made that cobalt may produce polycythemia by binding sulfhydryl or perhaps other groups active in cellular respirations, thus leading to a simulated cellular anoxia, in turn, to a compensatory polycythemia." Cobalt apparently does not affect the oxygen-carrying capacity of the erythrocyte (Suzuki, 1951). It is of interest to know that the polycythemic effect of cobalt in the rat can be inhibited by cysteine since it has been shown that selenium inhibits the maturation of erythrocytes in the duck and that cysteine inhibits this effect (Rigdon, 1953).

It is suggested from our studies in the duck that the polycythemia that occurs following the oral administration of cobalt may be explained by the direct action of this chemical on some enzyme system in the bone marrow. Such a mechanism apparently could produce a similar stimulating effect on the hematopoietic system as occurs when there is a diminution in the number of red cells resulting in anoxia.

The reticulocyte response of the duck to a hemolytic anemia as produced by phenylhydrazine hydrochloride and to the anemia produced by the withdrawal of blood is similar. The degree of response, as indicated by the number of reticulocytes, is much greater and occurs more quickly in the ducks receiving an adequate ration than in the birds receiving no food at the time when phenylhydrazine is given or blood is withdrawn. The nutritional factor necessary for the prevention of the fall in reticulocytes is present in casein. Further studies are necessary to determine whether a specific amino acid will influence the production of reticulocytes. The combination of eleven amino acids, as used in this study, did not significantly influence the production of reticulocytes.

It is of interest to find that vitamin $B_{12}$ and folic acid, both separately and together, do not prevent the rapid decrease in the number of reticulocytes in the peripheral blood when all foods are withdrawn from the duck. De Guia and Wolfred (1951) observed that a combination of folic acid and $B_{12}$ cured an anemia in chicks and suggested that $B_{12}$ may function somewhat as a catalyst by facilitating the uptake or release of folic acid by body

tissue. Nichol *et al.* (1949) also observed that $B_{12}$ did not have optimum activity on hemoglobin regeneration in chicks unless folic acid was present in adequate amounts. In the rat folic acid alone has been found to cause a rapid reticulocytosis (Bargoni, 1949). Apparently in the duck these two vitamins either separately or in combination do not affect the rapid diminution in reticulocytes that occurs when all foods are discontinued.

## *Summary*

The young duck is an excellent experimental host for the study of reticulocytosis because of the rapidity of growth and the presence normally of large numbers of reticulocytes within the peripheral blood. Marked changes may be produced in the number of reticulocytes in the peripheral blood of the duck within 72 hours.

Reticulocytes completely disappear from the peripheral blood within 72 hours after all foods are withdrawn from young ducks. These cells return to the peripheral blood within a similar period when food is replaced.

Ducks fed only sucrose or vegetable fat (Wesson oil) show a rapid diminution in the number of reticulocytes in the peripheral blood. Ducks fed only casein do not show a corresponding diminution in the number of reticulocytes in the peripheral blood.

The reticulocyte response to anoxia in the duck is much greater in the presence of an adequate diet than it is in ducks receiving no food.

It is suggested from these experiments that anoxia is the basic stimulus for the production of reticulocytes. This stimulus may result either from a deficient amount of oxygen reaching the bone marrow or through some failure in function of an enzyme system within the bone marrow. It is suggested that cobalt may produce the latter effect.

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# TRUE LATERAL HERMAPHRODITISM IN A PIG*

BERNARD L. YOLLICK†

True lateral hermaphroditism is a term used to denote the occurrence of male generative organs on one side of the body and of female on the opposite side. However, in general usage the term infers the presence of an ovary on one side of the body and a testis on the opposite side. There were no reports found of individuals possessing all the genital organs of both the male and female at the same time.

In a survey of the available literature only thirteen cases of true lateral hermaphroditism in pigs were found. As evidence of the rarity of this condition, Corner (1921) cites the systematic examination of 500,000 swine in the Berlin municipal abattoir by Pick in 1914. Only five cases of hermaphroditism were observed, and none were of the lateral variety, a mixed gland (ovotestis) on one or both sides being present in each. On the other hand Ostertag (1938) recorded six cases of true lateral hermaphroditism in pigs slaughtered at an abattoir in Karlsruhe, Germany. He believed the condition to be less rare than previously supposed.

## *Description*

The specimen was one of a number sent for study to the University of Alberta and subsequently forwarded[1] in a preserved state. It consisted of uterus, tubes, vagina, gonads, bladder, urethra and a large lymph node, from an adult pig (Fig. I).

The external genitalia were not complete in the specimen. The urethra traversed a small structure which resembled a penis. The external meatus was situated at the tip of this structure and was surrounded by a thickened prepuce. There was no glans. The vagina opened dorsicaudal to the penis-like structure.

The bladder was normal, lying ventral to the corpus uteri and narrowed to form a neck which continued into the urethra. On the right side of the

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[1] Graciously supplied by Swift Canadian Co. of Edmonton and forwarded by Professor R. Shaner of the University of Alberta to the Department of Anatomy, University of Saskatchewan.

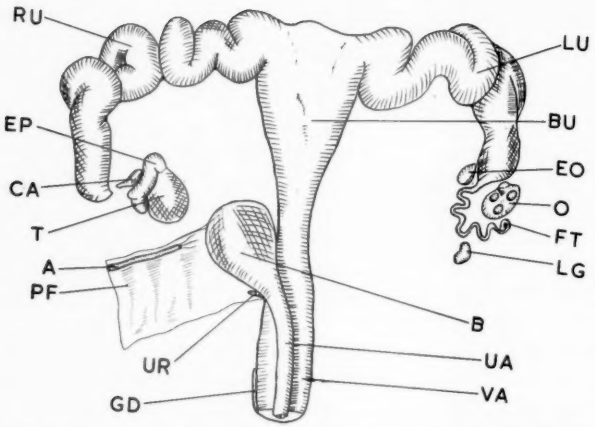


FIG. I. Genital organs of true lateral hermaphrodite pig. A, blindly-ending artery. B, bladder, BU, corpus uteri. CA, convoluted arteries. EO, remnant of Wolffian duct. EP, epididymis. FT, Fallopian tube. GD, Gartner's duct. LG, lymph gland. LU, left uterine cornu. O, ovary. PF, peritoneal fold. RU, right uterine cornu. T, testis. UA, urethra. UR, blindly-ending ureter. VA, vagina.

neck a small blindly ending conical tube projected upwards for about 2.5 cm. On section this proved to be a blindly ending ureter. Apparently there had been no connection between the right kidney and bladder at the time the animal was killed. The left ureter could not be found. It must be assumed that the latter had been separated from the bladder after the animal was slaughtered as there had to be adequate urinary excretion for the animal to have reached maturity. In a peritoneal fold reflected off the right side of the bladder a small blindly ending artery was found. This vessel had no connection with the bladder although a few minute branches came from it. A similar vessel has been observed in an intersexual sheep. On the left side of the bladder the peritoneum had been completely stripped off.

The uterus had two large cornua and a corpus uteri. Both cornua were dilated, thin walled and somewhat flexuous. The corpus uteri was thick walled. No definite differentiation could be observed between it and the cervix and vagina although the cervix was somewhat narrower than either the body of the uterus or the vagina. The extremity of the left cornu tapered to become continuous with the coiled Fallopian tube whose peritoneal orifice lay close to the ovary. On one side of this tube was an ovary and on the opposite a small grey lobulated structure which grossly resembled an epididymis but histologically appeared to be a remnant of the Wolffian duct such as the epoophoron. The ovary was small, rounded and contained a number of follicles, some of which projected from its surface. A large kidney-shaped lymph node was observed just caudal to the Fallopion tube.

The right uterine cornu ended abruptly, there being no Fallopian tube. A large oval-shaped testis was situated near the pointed blind end of the cornu. The outer surface of the testis was dark blue and its cut surface dark greyish brown. To its dorsal border and cephalic pole was attached a large epididymis with a blunt conical tail. There was no vas deferens. Dorsally the epididymis exhibited a mass of coiled arterioles as in the normal male. Opening at the right of the vaginal orifice was a narrow duct about one inch long, which according to its anatomical and microscopical appearance was a short Gartner's duct.

Histological examination of the testis showed normal configuration of the seminiferous tubules. Surrounding each tubule were numerous cells with small nuclei on a distinct basement membrane. The cytoplasm of these cells was drawn into narrow thread-like processes giving the lumen of the tubule a honeycomb appearance. They were probably Sertoli cells with some intermingling of poorly differentiated spermatogonia. There was no evidence of spermatogenesis. Between the tubules typical interstitial cells of Leydig were observed and some were heavily vacuolated. There was a large amount of interstitial connective tissue.

The histological appearance of the ovary was of a normal adult female. A few primitive follicles, one small corpus albicans and a large number of Graafian Follicles were observed. Some of the latter were undergoing involution towards corpora atretica. The stroma was typically cellular. In the hilus and medulla were some coiled arterioles. In histogical section the surface of the ovary appeared normal. In the numerous sections made, no testicular tissue could be found in the ovary and no ovarian tissue in the testis.

The epididymis-like structure on the left consisted of rather large tubules lined by pseudostratified ciliated columnar epithelium closely surrounded by very dense connective tissue which contained some blood vessels. The character of the loose connective tissue and the absence of smooth muscle and stereocilia made it appear unlike an epididymis and more like a rudiment of the Wolffian duct. On the right side, the epididymis appeared histologically normal. The uterus was lined by normal resting endometrium.

Histologic sections of the artery on the right of the bladder showed its elastica interna somewhat thickened and in some areas distinctly lamellated. In the adventitia was a peculiar structure surrounded by ossifying connective tissue. Elastic tissue stains showed it as a convolute of elastic fibres with a slit-like lumen, it being most probably an involuting artery. Apparently the main vessel had undergone slight elastosis similar to that in experimentally ligated vessels while the arteriole in the adventitia had undergone even more severe changes of the same nature.

*Discussion.* The literature points out certain fairly constant features with regard to the gonads in hermaphroditism of pigs. Whenever both ovarian and testicular tissue are present in the same animal, the gonads take the form of either an ovary with testis on the opposite side, an ovary with ovotestis on the op-

posite side, or two ovotestes. Further, where one gonad is an ovary it is almost always on the left side, and in an ovotestis the ovarian tissue is invariably cephalad to the testis. These observations led Crew (1924) to postulate an interesting explanation of the abnormalities of the gonads. He inferred that all these cases are genetically males and the sex determining factor complex located within the zygote included quickly elaborating female determining factors and slowly elaborating male determining factors. He further inferred that the differtiation of the gonads is not synchronous but consecutive, the left being affected before the right, and the cephalic pole before the caudal.

To explain true lateral hermaphroditism (Fig. II) it must be

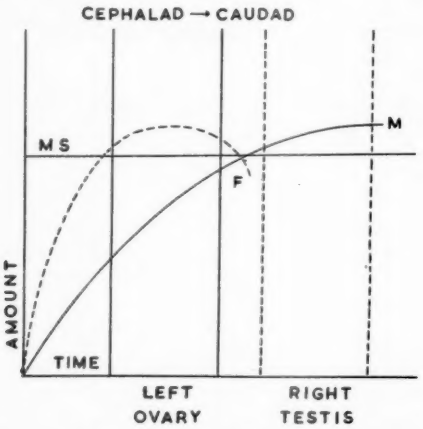


FIG. II. Redrawn and slightly modified after Crew. F, female sex determining factor. M, male sex determining factor. MS, minimum stimulus required for effective production of gonad.

assumed that the more quickly elaborated female determining factor has started the differentiation of the left gonad, influencing its full development into an ovary from cephalic to caudal pole. Subsequently the more slowly elaborated male determining factor had become in excess, and thus influenced the yet undifferentiated right gonad to develop into a testis. No satisfactory interpretation has been offered yet to explain the prevalent occurrence of ovarian tissue on the *left* side in cases of hermaphroditism.

*Summary*

1. True lateral hermaphroditism in the pig is comparatively rare.

2. A case is described in which the animal had an ovary on the left side and a testis on the right.

3. Crew's theory of hermaphroditism in pigs is used to explain the morphogensis of this case.

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# CALLING ATTENTION TO:

## Items of Possible Interest to Friends of Our Effort

January, 1955

General and Mental: A Allen & HL Mackinnon suggest use of psychologists & psychiatry social workers in supervised psychotherapy (*Dis Nerv Syst* 15/344/54) . . . Asso. Res. Nerv. Mental Diseases monographs *Genetics & Inheritance of Integrated Neurological & Psychiatric Patterns* (Williams & Wilkins. Balt, 54, 550pp, $10) . . . C Argyris studies *Executive Leadership: An Appraisal of a Manager in Action* (Harper, NY, 54, 154pp, $2.5) . . . E Bergler wittily analyses habit of not returning books (*Dis Nerv Syst* 15/340/54) . . . CS Bluemel offers *Psychiatry & Common Sense* (Macmillan, NY, 54, 259pp, $3) . . . Bd of Directors AAAS well proposes strengthening the basis of national security (*Science* 120/957/Dec 10/54) . . . HE Campbell & RG Livingstone discuss reducing auto injuries (*Surgery* 36/1056, 1059/54) . . . S & MT Chase describe *The Power of Words* (Harcourt, Brace, NY, 54, 320pp, $4) . . . HE Durkin offers *Group Therapy for Mothers of Disturbed Children* (CC Thomas, Springfield, Ill, 54, 125pp, $3) . . . F Fromm-Reichman approaches schizoid reactions as expression of & defense against anxiety (*Am J Psychiatry* 111/410/54) . . . I Galston eulogizes P Ehrlich (1854–1915) as biologist of vision (*Sci-month*, 79/395/54) . . . M Guttmacher considers tests for criminal responsibility (*Ibid* p 428) . . . GM Hochbaum studies relation of self-confidence to group pressure for conformity (*Am Soc Rev* 19/678/54) . . . A Lauterbach discusses *Man, Motives & Money: Psychological Frontiers of Economics* (Cornell Univ. Press, Ithaca, NY, 54, 366pp, $5) . . . JH Masserman gives conceptual dynamics of person, religion & self (*Phychoanal Rev* 41/303/54) . . . C Mayer goes *In Quest of New Ethics* (Beacon, Boston, 54, 334pp, $4) . . . J Needham offers 7 vols on *Science & Civilization in China* (Cambridge Univ Press, 54 & on, 300pp & 10 per vol) . . . PB Sears discusses human ecology as a problem in synthesis (*Science* 120/959/Dec 10/54) . . . B Ward offers a balance sheet of Western culture in *Faith & Freedom* (Norton, NY, 54, 308pp, $3.75) . . . W Weaver has no use for peace of mind (*Sat Rev* Dec 11/54, p 11) . . . N Wiener considers science, monkeys & Mozart, with comments on fears of scientific freedom (*Ibid* Nov 20/54) . . . FL Wright offers *The Natural House* (Horizon Press, NY, 54, 221pp, $6.5).

Basic Biology: HA Abramson & LT Evans find lysergic acid diethylamide tranquilizes Siamese fighting fish (*Science* 120/990/Dec 10/54) . . . R Altschul discusses *Endothelium: Its Development, Morphology, Function & Pathology* (Macmillan, NY, 54, 157pp, $3.5) . . . Our J Boss describes mechanism of anaphase (*Exp Cell Res* 7/443/54) . . . O Brenner-Holzach & J Raaflaub show swelling of isolated mitachondria depends on intramitachondrial concentration of ATP (*Helv Physiol Pharmacol Acta* 12/242/54) . . . F Buechner, E Letterer, & F Roulet edit 12 vol *Handbuch Allgemeinen Pa-*

*thologie* (Springer, Berlin, 54ff) . . . GR Cameron surveys exploration of cell in health & disease (*Brit Med J* 2/1061/Nov 6/54) . . . JM Chen describes organ culture in fluid media (*Exp Cell Res* 7/518/54) . . . HT Clarke edits *Ion Transport Across Membranes* (Academic Press, NY, 54, 298pp, $7.5) . . . SP Colowick & Co edit *Glutathione* (Academic Press, NY, 54, 341pp, $7.6) . . . E Ernst & J. Tigyi conclude muscle is not an osmotic sack (*Acta Physiol Acad Sci Hungarica* 6/145/54) . . . E Habermann & E Mollert show morphological differences in hemolysis from different drugs (*Arch Exp Path Pharmakol* 223/203/54) . . . S Kilpi & Co find 1% histodine sol maintains buffer capacity of blood after hemmorrhage (*Ann Chir Gyn Fenn* 43/143/54) . . . FE Lehmann & Co study inhibition of regeneration by amino-ketones (*Helv Physiol Pharmacol Acta* (12/147/54) . . . S Milkovic notes ascorbic acid Potentiates effect of ACTH on thymus (*Arch Internat Physiol* 62/309/54) . . . S Norton & Co show cyclizine ("maresine, BW") blocks histamine & vagi (*J Pharmacol* 112/297/54) . . . NB: Our CM Pomerat edits important symposium on *Tissue Culture Technique in Pharmacology* (*Ann NY Acad Sci* 58/971–1326/54) . . . E Racker edits Cellular Metabolism & Infections (Academic Press, NY, 54, 196pp, $4.8) . . . FS Sjostrand & V Hanzon describe membrane structure of cytoplasm, mitachondria & Golgi forms (*Exp Cell Res* 7/393, 415/54).

Clinical: AW Brothers & AE Bennett find mecholyl homeostasis test (DH Funkenstein & Co *Am J. Psychiatry* 106/889/50) aids in selecting patients for electrochock therapy (*Dis Nerv Syst* 15/335/54) . . . F Grosse-Brockhoff & Co discuss transposition of great vessels (*Deutsch Arch Klin Med* 20/305/54) . . . AG Macgregor & AR Sommer note p-amino salicylate in tuberculosis is followed by high incidence of goiter & hypothyroidism (*Lancet* 2/931/Nov 6/54).

### February, 1955

Scientists and Security: LM Terman shows scientists have basic personality differences from lawyers & business men, which may account for friction between scientists & government officials responsible for security clearances (*Sci Amer* 192/1/55) . . . MW Horowitz & N Pastore show relationship of motive to author & statement (*Science* 121/110/Jan 21/55) . . . AH Maslow discusses *Motivation & Personality* (Harper's NY, 54 411pp, $4.5) . . . Non-conformist B Russell considers *Human Society in Ethics & Politics* (Simon & Schuster, NY, 227pp, $3.5).

Basic: EG Boring appraises parapsychology (*Amer Sci* 43/108/55) . . . RJ Dubos indicates biochemical determinants of infection (*Bull NY Acad Med* 31/5/55) . . . LF Fieser reviews chemistry of steroids (*Sci Amer* 192/52/55) . . . GH Hitchings edits symposium on 6-mercaptopurine, an antimetabolite in leukemia (*Ann NY Acad Sci* 60/184–510/54) . . . H Kikhawa & Co describe pigments derived from tyrosine & tryptophan in animals (*Science* 121/43/Jan 14/55) . . . JI Kitay & MD Altschule review the physiology of *The Pineal Gland* (Harvard Univ Press, Cambridge, Mass, 54, 280pp, $4) . . . F Lipmann discusses relation of coenzymes to biosynthesis (*Amer Sci* 43/37/55) . . . EW McChesney & JO Hoppe find iodopanoic acid, a useful

oral cholecystographic agent, excreted as glycuronide without reabsorption (*Arch Internat Pharmaco* 99/127/54) . . . WD McElroy & B Glass edit symposia on *Amino Acid Metabolism* (Johns Hopkins Press, Balt, 55, 1045 pp, $12.5) and on *The Mechanism of Enzyme Action* (*Ibid*, 54, 819pp, $11) . . . HJ Muller discourses on life as a consequence of the mode of organization of matter (*Science* 121/1/Jan 7/55) . . . VA Najjar edits symposium on *Fat Metabolism*, Johns Hopkins Press, Balt, 54, 194pp, $4.5) . . . FH Netter offers great color illustrations for *Nervous System* and for *Reproductive System*, vols 1 & 2 in the *Ciba Collection of Medical Illustrations* (Ciba Pharmaceutical Products, Summit, New Jersey, 54, 145pp, $6, & 55, 286pp, $13) . . . RW Schayer & Co find cortisone inhibits histamine reactions in tissues *Proc Soc Exp Biol Med* 87/590/54) . . . JA Schneider finds reserpine antagonises morphine analgesis, while chloropromazine enhances it (*Ibid*, p 614) . . . ME Shils & WB Stewart find tryptophan & lysine prevent fatty liver (*Ibid*, p 629) . . . L Szege notes antihyaluronidase action of p-amino salicylate (*Acta Physiol Acad Sci Hungar* 6/371/54) . . . S Wawzonek & Co find aminonitriles cause epiphyseal plate lesions, degenerative arthritis, & aneurysm of the aorta (*Science* 121/63/Jan 14/55) . . . BH Willier, PA Weiss & V Hamburger edit *Analysis of Development* (Saunders, Phila, 55, 750 pp, $7).

CLINICAL: A Buhlmann & P Luchsinger review problems of artificial respiration (*Schwei Med Wchnschr* 85/1/Jan 1/55) . . . DB Cheek gives survey of fluid & electrolyte balance in disease (*Med J Austral* 2/917/Dec 11/54) . . . D Hubble emphasizes pituitary in endocrine relations (*Lancet* (1/1/Jan 1/55) . . . AE Hussar & HL Holley give a clinical summary of *Antibiotics & Antibiotic Therapy* (Macmillan, NY, 54, 475pp, $6) . . . HT Hyman offers *Handbook of Treatment* (Lippincott, Phila, 55, 511pp, $8) . . . JR Jaenike & C Waterhouse note dextran prolongs bleeding time (*Circulation* 11/1/55) . . . WH Masters & JW Ballew propose mixed estrogens & androgens for handling senile neuters (*Geriatrics* 10/1/55) . . . DJ McCarthy & KM Corrin suggest *Medical Treatment of Mental Disease: The Toxic & Organic Aspects of Psychiatry* (Lippincott, Phila, 55, 653pp, $12) . . . R Patala & Co show synthetic detergent solutions are non-irritating & optimal for use in concentrations of 0.2% (*Ann Chir Gyn Fenn* 43, Suppl 5, 54, 60th birthday festschrift for Aleksander R. Klossner, 332pp) . . . DD Reid suggests design for clinical experiments (*Lancet* 2/1293/Dec 25/54) . . . HT Ricketts discusses *Diabetes Mellitus: Objectives & Methods of Treatment* (CC Thomas, Springfield, Ill, 55, 123 pp, $3.5).

GENERAL: S Esche offers critical catalogue in *Leonardo da Vinci: Das Anatomische Werk* (Holbein-Verlag, Basel, 54, 174pp, $13) . . . I Galdston introduces symposium on Paul Ehrlish (1854–1915) with comments by O Temkin, E Jokl & CP Rhoads (*Bull NY Acad Med* 30/953–987/54) . . . E Sachs delights in *Prerequisites of Good Teaching* (Shoe String Press, Hamden, Conn, 54, 118pp, $1.5).

# PHENYLSERINE STUDIES. I. A SURVEY OF RING-SUBSTITUTED PHENYLSERINE ANALOGUES*

ERNEST BEERSTECHER, JR., ELEANOR J. EDMONDS, AND CAROL M. VOLKMANN

In 1894 Erlenmeyer reported that hydrolysis of one of the products resulting from the condensation of glycine and benzaldehyde yielded a synthetic amino acid which he termed *β-phenylserine*. It was over fifty years, however, before the significance of this compound was fully realized. In the past decade phenylserine derivatives, prepared by the Erlenmeyer reaction from substituted benzaldehydes, have been shown to be of direct interest in the biosynthesis of adrenaline and noradrenaline, to undergo autooxidation and condensation to form derived melanins, to be bacteriostatic and, indeed, to be the parent compounds of chloromycetin. There is some suggestion, moreover, that appropriate derivatives may be discovered which will manifest an inhibitory action against cancer. In view of these considerations, a survey has been made of the general adaptability of the Erlenmeyer reaction, and a large number of phenylserine derivatives has been prepared. The present paper summarizes existing knowledge concerning the phenylserines, and demonstrates the simplicity of preparation of numerous analogues. Subsequent publications will deal in detail with the synthesis and biological properties of these substances.

## *Historical*

*Synthesis.* The original Erlenmeyer condensation (1894) was performed in the presence of alcohol and strong sodium hydroxide, and the intermediate product hydrolyzed with acetic acid. Optimal conditions for large scale preparation are described in a German patent (1936), and for the nitrophenylserines in a second patent (1952). Most subsequent workers have obtained somewhat better overall results by working with glycine esters and hydrolyzing with hydrochloric acid (Rosenmund and Dornsaft, 1921; Hartung, 1953). A variety of other more arduous methods of synthesis have also been employed in order to obtain special phenylserine analogues. Phenylserine contains two asymmetric carbon atoms and can thus exist in *erythro-* and *threo-*

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forms. Reduction of phenylserine with lithium aluminum hydride results in *threo*-phenylserinol (Carrara and Weitnauer, 1949).

Conversion without inversion to phenylglyceric acid results in the *threo*-form of this latter compound (Billet, 1950a). This form of phenylserine is active against bacteria, as is the *threo-* (*trans*) form of chloromycetin, while the *erythro-* form of the latter is not bacteriostatic. It thus appears that *threo*-phenylserine is the isomer produced by the Erlenmeyer condensation (Vogler, 1950). It has been reported that condensations in neutral medium, in contrast to the alkaline conditions usually employed, produce the *erythro-* form (*Kopp et al.*, 1951). It is apparent that derivatives of phenylserine may not always behave uniformly in this regard, since *p*-nitrophenylserine prepared by condensation is not bacteriostatic and is the *erythro-* form, while that prepared by nitrating phenylserine is *threo-* and it is inhibitory (Billet, 1950b; Molho and Molho-Lacroix, 1951; Holland and Jenkins, 1951; Elphimoff-Felkin *et al.*, 1951, 1952ab; Fones, 1952, 1953). Recent workers are tending to restrict the term *phenylserine* to the *threo-* form and to refer to the *erythro-* form as *allophenylserine*.

Table 1 summarizes the ring-substituted analogues of phenylserine that have been reported in the literature. Attempts to prepare hydroxy-amino acids

TABLE 1

*Reported Analogues of Phenylserine*

| Analogue | Mpt. | Reference |
|---|---|---|
| phenylserine (D,L, *erythro-*, *threo-*) | 190° | Erlenmeyer and Früstück, 1894 |
| *p*-nitro- (*erythro-*, *threo-*) | dec. 188° | Dalgleish, 1949 |
| 3,4-dihydroxy- | 219–21° | Rosenmund and Dornsaft, 1919 |
| *p*-hydroxy- | dec. 217° | Rosenmund and Dornsaft, 1919 |
| *p*-methoxy- | hydrated needles 185–6° | Rosenmund and Dornsaft, 1919 |
| 3-methoxy-4-hydroxy- | dec. 195° | Rosenmund and Dornsaft, 1919 |
| O-carbethoxy-*p*-hydroxy-,HCl salt | 181° | Rosenmund and Dornsaft, 1921 |
| *o*-methoxy- | dec. 227–32° | Forster and Rao, 1926 |
| *p*-amino- | dec. 205–7° | Dalgleish, 1949 |
| *m*-nitro- | 203° | Bergmann *et al.*, 1950 |
| 2,6-dichloro- , HCl salt | 198° | Bergmann *et al.*, 1950 |
| 3,4-methylenedioxy- | 200–1° | Kanao & Shinozuka, 1947 |
| *p*-chloro- | .. | Hartung *et al.*, 1953 |
| *m*-amino- | .. | Bergmann *et al.*, 1953 |

from aliphatic aldehydes have apparently not been successful (Rosenmund and Dornsaft, 1921), although aliphatic hydroxy-amino acids are subject to decomposition to form glycine and the corresponding aldehyde or ketone (see below). Efforts to synthesize analogues from the following benzalde-

hyde derivatives have also been reported unsuccessful: 3,4-dimethoxy- (Mann and Dalgliesh, 1946); *p*-dimethylamino-, 2,4-dinitro-, 3,4-dinitro-, *p*-benzoxy-, *p*-methoxy- (Bergmann *et al.*, 1951). The first and last of these, however have been prepared, as indicated in the experimental section of this present paper.

*Chemical Properties.* The phenylserines have the general chemical properties of other alpha amino acids, and numerous esters and amino derivatives have been prepared. Peptides have been prepared from phenylserine itself (Doherty *et al.*, 1943). A unique property of the phenylserines is their mode of decomposition. Carter (1938) showed that when heated with HBr, two molecules of phenylserine tend to condense to form $\beta$-phenylnaphthalene. Of more general importance, however, is the natural tendency of the condensation by which these amino acids are formed to reverse itself.

Regardless of how thoroughly they are purified, phenylserines always retain the odor of the benzaldehyde from which they were prepared due to this reverse aldol tendency. Refluxing of phenylserine with 10 per cent sodium hydroxide converts it to benzaldehyde and glycine (Bettzieche, 1925), and this fact may be utilized in determining phenylserine in biological mixtures containing negligible amounts of other hydroxy-amino acids. Distillation of phenylserine at 32 mm. yields benzaldehyde (Fourneau and Billeter, 1940). Methylation of phenylserine, as well as serine, threonine, hydroxyaspartic and hydroxyglutamic acid, yields significant amounts of betaine (Dakin, 1941). A glycinogenase prepared from guinea pig liver converts phenylserine to benzaldehyde, hydroxyvaline to acetone, and threonine and allothreonine to acetaldehyde (Vilenkina, 1949). The enzyme is inhibited by carbonyl reagents, but not by vitamin $B_6$ deficiency. This latter fact is of interest in view of the fact that pyridoxal, in the presence of copper, iron or aluminum salts, converts threonine or allothreonine and serine to glycine plus acetaldehyde or formaldehyde respectively (Metzler *et al.*, 1953). Presumably phenylserine would react similarly in this artificial system.

*Metabolism.* The first studies on the metabolism of phenylserine were those of Dakin (1909), who found that when 1.5 gm. of the amino acid were fed to a cat it was possible to recover 0.9 gm. of hippuric acid from the urine. It is apparent that following cleavage of the amino acid to form benzaldehyde, the latter compound was oxidized to benzoate and coupled to glycine. Further studies of the reaction were conducted by Vilenkina (1949), using enzyme preparations from guinea pig liver. The enzyme involved, glycinogenase, was found to act upon all $\beta$-hydroxy-amino acids, converting them to glycine and the appropriate aldehyde or ketone. The reaction was inhibited by carbonyl reagents, but not by vitamin $B_6$ deficiency, and this latter fact appears to differentiate the reaction from the non-enzymatic, pyridoxal-mediated cleavage of Metzler *et al.* (1953).

Werle and Peschel (1949) observed that guinea pig liver and kidney slices oxidize phenylserine and *p*-hydroxphenylserine at a pH range of 9.0 to 9.4, but not at 8.0. Tissue extracts, however, attack only the *p*-hydroxyphenylserine, suggesting that two enzyme systems are involved. In this case, however, the extracts are even more active than the slices. The oxidation is not

accompanied by deamination. Using *Crotalus adamanteus* venom as an enzyme source, Fones (1952) found that DL-*threo*-phenylserine was converted to D-mandelic acid and DL-*erythro*-phenylserine was converted to L-mandelic acid. Hog kidney D-amino acid oxidase was apparently inactive. It was subsequently found (Fones, 1953) that the *erythro*- D- and L- forms were acted upon by D- and L- amino acid oxidases respectively, but that the *threo*- forms were not acted upon to any measurable degree.

Rosenmund and Dornsaft (1919, 1921) were the first to suggest that 3,4-dihydroxyphenylserine might be the parent compound of adrenaline, but their theory was discarded by Knoop (1919). Subsequent studies by Mann and Dalgliesh (1946) and others on N-methyl-3,4-dihydroxyphenylserine have reopened this question. Blaschko and co-workers (1948, 1950) have shown that 3,4-dihydroxyphenylserine is not a substrate for dopa decarboxylase, that the latter enzyme is not present in guinea pig adrenal gland extracts, and that these extracts do not decarboxylate the amino acid. On the other hand, guinea pig kidney under anaerobic conditions does decarboxylate the amino acid to form noradrenaline. Only the L isomer is attacked, and the N-methyl derivative is not affected. Werle and Peschel (1949) obtained similar results with guinea pig kidney, and with beef kidney, adrenal and pancreas, and reported that the activity was stimulated by ascorbic acid. Sourkes *et al.* (1952) found that both 3,4-dihydroxyphenylserine and 3,4-DOPA were decarboxylated by the medulla of beef adrenal glands. Schmiterlow (1951) studied the reaction with rabbit tissues, and Blaschko *et al.* (1948) obtained active acetone dried extracts of *Streptococcus fecalis*. Since 3,4-dihydroxyphenylserine is not known to occur naturally, however, there remains considerable doubt as to whether this is the natural mode of formation of noradrenaline.

Armstrong and Lewis (1950) reported that phenylserine did not support the growth of rats, and that it was not converted in the rat to either phenylalanine or serine. Subsequently Shaw and Armstrong (1954) have demonstrated this to be true for the *erythro*- and *threo*- forms individually.

*Enzyme Inhibition.* Beerstecher and Shive (1946) first reported that DL-phenylserine was inhibitory to the growth of bacteria (***Escherichia coli*, *Lactobacillus arabinosus*** 17-5, and *Streptococcus faecalis* R). The inhibition was associated with enzymatic processes in phenylalanine and tryptophan metabolism, since either of these two amino acids was able to reverse the effect. Neither phenylpyruvic acid nor tyrosine affect the inhibition (Beerstecher and Shive, 1947a,b) so that it was concluded that phenylserine inhibits the conversion of phenylalanine to some as yet unidentifiable metabolite necessary for growth. Fox and Warner (1954) have recently analysed the effects of the various isomers of phenylserine, with and without added phenylalanine, on *Lactobacillus arabinosus* 17-5 and *Lactobacillus brevis*. Martin and Moss (1949) reported that phenylserine inhibited the growth of *Pseudomonas aeruginosa*.

The discovery of chloromycetin at this time, and its obviously close structural relationship to phenylserine, stimulated intensive studies in this area. Mentzer *et al.* (1950a,b) reported that DL-*threo*-phenylserine inhibited *Escherichia coli*, and that its action was augmented by L-aspartic acid and

diminished by glycine. Billet (1950a,b,c, 1951, 1952) studied the *p*-nitro analogue of phenylserine and concluded that, as in chloromycetin, only the *threo*- isomer is active. The isomer obtained by the Erlenmeyer reaction is inactive. Similar results were found by Molho and Molho-Lacroix (1950, 1951). Marnay (1951) has reported that the phenylserine inhibition of *Escherichia coli* is reversed by niacin, a finding not in accord with the earlier works of Beerstecher and Shive. Molho-Lacroix and Molho (1952a) found that chloromycetin-resistant strains of *Escherichia coli* became more sensitive to phenylserine. These authors (1952b) also found that phenylserine inhibits the conversion of phenylalanine to tyrosine, but that after the organism becomes adapted to phenylserine, it developes the ability to synthesize tyrosine directly without the utilization of phenylalanine as an intermediate. Bergmann *et al.* (1953) found that *m*- and *p*- nitro- and aminophenylserine counteract the phenylserine inhibition of *Escherichia coli*, but are inhibitory in themselves. Smith (1952), studying the effects of chloromycetin culture decomposition products, found that *p*-nitrobenzaldehyde and *p*-nitrophenylserine stimulated the growth of *Escherichia coli*, and that the former compound reversed chloromycetin inhibition.

*Experimental and Discussion.* The present study was limited to a survey of the original experimental conditions described by Erlenmeyer. 5 mM. glycine (.37 gm.) dissolved in 2.0 ml. water, 10 mM. (1.–2. gm.) of the appropriate benzaldehyde derivative, 1.0 ml. 95 % ethyl alcohol, and 2.0 ml. of 25% aqueous sodium hydroxide were introduced into a six-inch test tube. The mixture was stirred with a glass rod for about one-half hour, and then just acidified with glacial acetic acid. A small portion of the crude condensation mixture was added to the bottom of a large sheet of Whatman No. 2 filter paper, and chromatographed in a 4:1:1 mixture of n-butanol-ethanol-water. The dried chromatograms were sprayed with ninhydrin solution, and the sheets heated in an oven at 98° C. Since the synthesis permits the formation of only one kind of amino acid in each case, the presence of an amino acid spot on the sheet other than that of the unreacted glycine shows that reaction occurred, and the size of the spot indicates the relative yield that was obtained. In some instances, atypical brown and yellow spots were obtained due to the presence of unreacted benzaldehyde, undecomposed imino derivatives, or possible side reactions. Table 2 lists the Rf values and relative yields for some forty-two derivatives, and Figure I illustrates the appearance of a typical chromatogram.

The general reactivity of the various benzaldehyde derivatives follows well what might be anticipated on the basis of the rela-

TABLE 2

*Summary of Reactivity of Ring-Substituted Benzaldehydes and Rf Values of Phenylserine Derivatives*

| Phenylserine Derivative | Rf Values Bu-EtOH-$H_2O$ | Yield of Analogues* |
|---|---|---|
| Phenylserine | .41 | ++ |
| *Phenolic Analogues* | | |
| 2-Hydroxy- | .25 | ++ |
| 3-Hydroxy- | .. | — |
| 4-Hydroxy- | .. | — |
| 2,4-Dihydroxy- | .31 | ++ |
| 3,4-Dihydroxy- | .. | — |
| *Alkoxy- Analogues* | | |
| 2-Methoxy- | .48 | +++ |
| 4-Methoxy- | .40 | ++ |
| 2-Ethoxy- | .58 | +++ |
| 2,3-Dimethoxy- | .60 | +++ |
| 3,4-Dimethoxy- | .25 | ++ |
| 3,4-Methylenedioxy- | .36 | ++ |
| 3,4-Diethoxy- | .63 | ++ |
| 2-Hydroxy-3-methoxy- | .30 | + |
| 4-Hydroxy-3-methoxy- | .. | — |
| 4-Hydroxy-3-ethoxy- | .. | — |
| *Alkyl-Aryl-Analogues* | | |
| 3-Methyl- | .42 | ++ |
| 4-Methyl- | .42 | ++ |
| 4-Isopropyl- | .65 | + |
| 4-Phenyl- | .58 | + |
| *Halide Analogues* | | |
| 2-Fluoro- | .27 | +++ |
| 3-Fluoro- | .38 | ++ |
| 4-Fluoro- | .37 | ++ |
| 2-Chloro- | .51 | +++ |
| 4-Chloro- | .63 | +++ |
| 2,4-Dichloro- | .73 | +++ |
| 3,4-Dichloro- | .75 | +++ |
| 5-Chloro-2-hydroxy- | .62 | +++ |
| 5-Bromo-2-hydroxy- | .63 | +++ |
| *Nitro Analogues* | | |
| 2-Nitro- | .33 | + |
| 3-Nitro- | .45 | +++ |
| 4-Nitro- | .50 | +++ |
| 2,4-Dinitro- | .. | — |
| 2-Hydroxy-5-nitro- | .. | — |
| *Amino Analogues* | | |
| 2-Amino- | .. | — |
| 4-Amino- | .. | — |
| 4-Dimethylamino- | .. | — |
| 4-Diethylamino- | .. | — |
| 4-Succinylamino- | .. | — |
| *Sulfonate Analogues* | | |
| 3-Sulfonyl-4-hydroxy- | .. | — |
| 3-Sulfonyl-4-methoxy- | .. | — |
| *Miscellaneous* | | |
| Pyridoxyl- | .. | — |
| Furyl- | .35 | +++ |

* — = none, + = moderate, +++ = large.

tive electrophilic properties of the ring substituents and their consequent activating or deactivating effects on the aromatic aldehyde group. These theoretical considerations, however, are modified somewhat by solubility differences, stability to pH, and side reactions. Hydroxybenzaldehydes, for example, uniformly undergo oxidation and condensation in the reaction, to form black melanin-like substances. Bearing these factors in mind, it may nevertheless be stated that halogen-substituted benzaldehydes are uniformly highly reactive, alkoxy- derivatives only slightly less so, and alkyl- compounds moderately active. Aminobenzaldehydes, whether substituted on the amino group or not, are apparently completely unreactive under the conditions employed.

Modification of the pH of the condensation, or the use of glycine ester in place of glycine itself, might be expected to improve materially the yields in many instances. Isolation of the individual analogues may generally be achieved by washing the crude mixture with alcohol and ether, and recrystallizing the relatively insoluble analogues from water as described by Erlenmeyer. Detailed methods will be described in subsequent publications. Preliminary investigations show that most of the analogues do not possess high biological activity, suggesting that they are for the most part in the *erythro-* form.

## *Summary*

The literature relating to ring-substituted phenylserine analogues has been reviewed. The adaptability of the Erlenmeyer reaction to the preparation of forty-two ring-substituted derivatives of this amino acid has been studied by means of paper chromatography, and variables relating to the reaction have been discussed.

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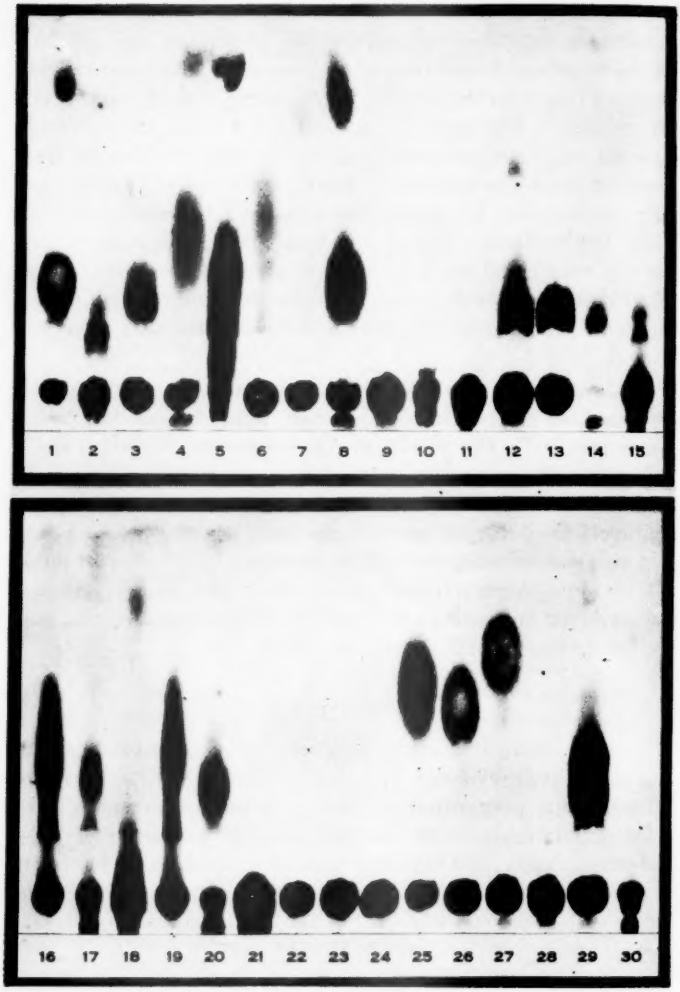


Fig. I. Paper chromatograms of crude phenylserine reaction mixtures. 1- phenylserine, 2- *o*-hydroxy-, 3- *p*-methoxy-, 4- *o*-ethoxy-, 5- *o*-chloro-, 6- 3,4-diethoxy-, 7- *p*-isopropyl-, 8- *o*-methoxy-, 9- *p*-hydroxy-, 10- 2-hydroxy-5-nitro-, 11- 4-hydroxy-3-sulfonate, 12- 3,4-dimethoxy-, 13- 3,4-methylenedioxy-, 14- *o*-nitro-, 15- 2-hydroxy-3-methoxy-, 16- 5-chloro-2-hydroxy-, 17- *p*-nitro-, 18- 2,4-dihydroxy-, 19- 5-bromo-2-hydroxy-, 20- *m*-nitro-, 21- 4-methoxy-3-sulfonate, 22- *p*-dimethylamino-, 23- *m*-hydroxy-, 24- *p*-diethylamino-, 25- 2,4-dichloro-, 26- *p*-chloro-, 27- 3,4-dichloro-, 28- 3-methoxy-4-hydroxy-, 29- 2,3-dimethoxy-, 30- pyridoxyl-.

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# ANTIGENIC STUDIES ON THE PSITTACOSIS-LYMPHOGRANULOMA GROUP OF VIRUSES

## I. Acid-soluble Complement-fixing and Skin Test Antigens*

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The infective agents belonging to the psittacosis-lymphogranuloma venereum (LGV) group are antigenically closely related. However, strains of these viruses can be differentiated by their pathogenecity spectra, infectivity and toxin neutralization tests. Bedson (1936) has shown that by using special procedures these viruses contain type specific as well as group specific complement-fixing (CF) antigens. Also, psittacosis and LGV skin test antigens extracted with dilute acid were shown to be specific when tested in a small number of human patients (Barwell, 1952). Despite these findings it is not possible on a practical basis to make a definitive diagnosis between psittacosis and LGV by the CF reaction.

Thus, attempts were made to extract and partially purify skin test and CF antigens from members of the psittacosis-LGV group, so that they may be studied for their possible antigenic specificities and biologic properties. This report deals with preliminary studies on the antigen reported by Barwell (1952) to be specific as a skin test reagent.

*Methods.* Psittacosis (Texas turkey) (Pst), meningopneumonitis (Francis) (MP), LGV (JH), feline pneumonitis (Baker) (FeP), and mouse pneumonitis (Nigg II) (MoP) viruses were used in this study.

Skin test antigens were made in a manner similar to the technique given by Barwell (1952). Yolk sac inoculations of 6 or 7 day old embryos were made with either psittacosis, LGV, FeP, MP, or MoP viruses. The eggs were incubated at 35–36° C.,

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and the yolk sacs harvested when the embryos died 4-5 days later. The sacs were collected in 2 ml. of saline per sac and shaken with glass beads for 30 minutes. The suspension was centrifuged at 2,000 rpm, for 10 minutes and the resulting supernatant fluid centrifuged at 30,000 x G for 30 minutes. The pellet from this centrifugation was suspended in saline at one-half of the original volume of sac suspension and cleared at 2,000 rpm. for 10 minutes. To this virus suspension 0.1 N HCl was added to give a final concentration of 0.02 N HCl. The acid suspension was incubated at 37° for 20 minutes and then centrifuged at 30,000 x G for 20 minutes. The supernate was decanted and upon adjustment to pH 7.4 with 0.1 N NaOH an occasional fine precipitate formed at about pH 4.5. The precipitate was removed by centrifugation and discarded.

Psittacosis, MP and FeP viruses were also propagated in chick embryos by allantoic inoculation. The viruses in the allantoic fluids were partially purified by 3 cycles of differential centrifugation according to the technique given previously (Benedict, 1955). Virus obtained by this method was extracted in a manner similar to that described with yolk sac grown virus. It was noted, however, that upon neutralization of the acid extract with NaOH a voluminous precipitate formed at about pH 4.0–4-5. The precipitate was removed by centrifugation, washed three times in saline (pH 7.0) by centrifugation and re-suspension, and was saved. The precipitate was amphoteric as it was soluble below pH 5.0 and above pH 8.5. It was used in the insoluble state; that is, suspended in saline at pH 7.0. After removal of this precipitate, the supernate was cleared by centrifugation at 30,000 x G for 20 minutes and saved for studies.

The group CF antigen was prepared by the usual procedure of heating allantoic fluid infected with psittacosis virus for 30 minutes at 100° C. Preservation was made with 0.5 per cent phenol.

Groups of albino rabbits were inoculated either intracerebrally or intraperitoneally with either mouse-propagated psittacosis, MP, LGV, FeP or MoP viruses. Forty days following inoculation their CF titers were determined, and they were subsequently skin tested with the acid extracted antigens made from

yolk-sac grown viruses. Serving as a control, Lygranum[1] was also given intradermally.

Groups of white guinea pigs were infected with psittacosis grown in mouse brain. The $LD_{50s}$ of the inocula, as determined by intracerebral mouse inoculation, varied from $1.5 \times 10^1$ to $1.5 \times 10^5$. Guinea pigs were found to be skin sensitive 4 weeks following the infecting inoculation.

The flanks of the animals were shaved with electric clippers and inoculated with 0.1 ml. of the skin test reagents. The skin tests were observed for immediate reactions and the diameters of the erythema and induration of the 24 and 48 hour reactions were measured in mm. and recorded. The height of the reactions were observed to be 24 hours following the test.

For the CF test, sera[2] were inactivated at 60° C, for 20-30 minutes. The preliminary incubation of the CF test was carried out at 37° C for 75 minutes, and after the addition of sensitized sheep erythrocytes, a secondary incubation at 37° C for 30 minutes was made. Titers represent the reciprocal of the highest dilution showing a 4+ reaction.

## *Results*

*Skin Tests with acid extracts.* The results of the skin testing of rabbits with yolk-sac antigens are summarized in table 1. Rabbits inoculated with FeP and MoP viruses were not infected as shown by their failure to develop either detectable complement-fixing antibodies or allergic cutaneous reactions. It was obvious however that rabbits infected with psittacosis, MP and LGV viruses reacted only to their homologous antigens and to the Lygranum control. These results, in which experimental animals were used, corroborate the observations of Barwell (1952).

Guinea pigs infected with psittacosis for 5 weeks were skin tested with the precipitate and supernatant fractions obtained from allantoic fluid acid extracts. A typical experiment is summarized in table 2. The insoluble precipitate and supernate of the psittacosis acid extract were active; however, it has been repeatedly observed that the precipitate contained the greatest al-

[1] Lygranum S. T.: E. R. Squibb & Sons, New York.

[2] Some of the LGV sera were generously supplied by Dr. Morris F. Shaffer, School of Medicine, Tulane University, New Orleans, La.

TABLE 1

*Skin Test Activity of Acid-Extracted Antigens (yolk sac) in Rabbits Infected with Psittacosis-LGV Viruses*

| Infected with | Lygranum | Skin test reagent Pst | MP | LGV | FeP | MoP |
|---|---|---|---|---|---|---|
| Pst .... | 5 x 5* | 15 x 15 | 0 | 0 | 0 | 0 |
| Pst .... | 8 x 6 | 25 x 25 | 0 | 0 | 0 | 0 |
| MP ... | 14 x 14 | 0 | 10 x 10 | 0 | 0 | 0 |
| MP ... | 3 x 3 | 0 | 4 x 4 | 0 | 0 | 0 |
| MP ... | 9 x 8 | 0 | 15 x 15 | 0 | 0 | 0 |
| MP ... | 4 x 4 | 0 | 15 x 23 | 0 | 0 | 0 |
| LGV ... | 10 x 10 | 0 | 0 | 8 x 6 | 0 | 0 |
| LGV ... | 6 x 6 | 3 x 3 | 0 | 15 x 10 | 0 | 0 |
| LGV ... | 0 | 0 | 0 | 0 | 0 | 0 |
| LGV ... | 3 x 3 | 0 | 0 | 4 x 4 | 0 | 0 |
| *Normal* | | | | | | |
| 1 ..... | 0 | 0 | 0 | 0 | 0 | 0 |
| 2 ..... | 2 x 2 | 0 | 0 | 0 | 0 | 0 |
| 3 ..... | 0 | 0 | 0 | 0 | 0 | 0 |

* Diameters in MM ; 24 hr. reading.

TABLE 2

*Activity of Psittacosis and Meningopneumonitis Acid-Extracted Skin-Test Antigens (allantoic) in Guinea Pigs Infected with Psittacosis*

| Guinea Pig | Antigen psittacosis Precipitate | psittacosis Supernate | meningopneumonitis Precipitate | meningopneumonitis Supernate |
|---|---|---|---|---|
| 1 ..... | 5 x 5* | 0 | 5 x 5 | 0 |
| 2 ..... | 8 x 4 | 0 | 5 x 5 | 0 |
| 3 ..... | 4 x 4 | 0 | 4 x 4 | 0 |
| 4 ..... | 10 x 10 | 2 x 2 | 8 x 8 | 3 x 3 |
| 5 ..... | 15 x 10 | 15 x 16 | 15 x 6 | 10 x 10 |
| *Normal* | | | | |
| 1 ..... | 0 | 0 | 0 | 0 |
| 2 ..... | 0 | 0 | 0 | 0 |
| 3 ..... | 0 | 0 | 0 | 0 |

* Diameters in MM ; 24 hr. reading.

lergenic activity. The acid extract of virus preparations processed from allantoic fluid failed to show the same specificity as did the extracts obtained from virus grown in the yolk sac. As shown in table 3, FeP acid extract (precipitate) detected cutaneous hypersensitivity in guinea pigs infected 6 weeks with psittacosis. This lack of specificity when compared to the specific reactions obtained in rabbits is as yet unexplained.

*Complement-fixation tests with acid extracts.* Although Barwell (1952) failed to find CF activity with the acid extracts, our preparations (allantoic) were tested for this property. When

TABLE 3

*Activity of Acid Extracted Skin Test Antigens in Guinea Pigs Infected with Psittacosis*

| Guinea Pig | Antigen | | |
|---|---|---|---|
| | Pst | MP | FeP |
| 1 | 15 x 15* | 9 x 9 | 3 x 3 |
| 2 | 19 x 19 | 6 x 6 | 0 |
| 3 | 7 x 7 | 3 x 3 | 2 x 2 |
| 4 | 12 x 10 | 11 x 10 | 7 x 7 |
| 5 | 20 x 20 | 17 x 12 | 15 x 12 |
| 6 | 17 x 14 | 13 x 13 | 10 x 10 |
| *Normal* | | | |
| 1 | 0 | 0 | 0 |
| 2 | 0 | 0 | 0 |
| 3 | 3 x 3 | 0 | 0 |

* Diameters in MM ; 24 hr. reading.

extremely large quantities of virus were extracted with either 0.02 or 0.05 N HCl at 37° or 60° C, the extract was capable of fixing complement in the presence of specific antiserum. The activity resided in the precipitate fraction that formed upon neutralization of the acidic extract. The possibility that residual particles might account for the CF activity was ruled out by clearing the acid precipitate in its soluble state (pH 4.0) at 130,000 x G for 45 minutes. No change in CF activity resulted from this treatment. Antigen prepared from psittacosis virus detected psittacosis antibody from different animal species; however, the same concentration of antigen was only weakly active for the detection of LGV antibody in human sera (Table 4). For comparison purposes, 4 units of both the standard heated group and acid-extracted antigens were used. Further substantiating this specificity, an acid extract of feline pneumonitis failed to detect psittacosis antibody.

*Discussion.* The data presented here indicate that when employing the acid extracted antigen, the skin test is valuable for the differentiation of some of the psittacosis-LGV strains in experimental animals. The exact conditions under which identification of these agents may be made possible by this procedure are not as yet well defined. Acid extracts made from yolk sac virus preparations were strain specific when tested in rabbits, and MP, which has been considered a psittacosis strain, failed to cross react with the psittacosis virus we had employed. Similar acid extracts made from virus obtained from allantoic fluid failed

TABLE 4

*Complement-Fixing Activity of the Psittacosis Acid Extract as Tested with Psittacosis and Lymphogranuloma Venereum Antisera*

| Serum | Antigen | Serum Titer |
|---|---|---|
| Human pst | G* | 20 |
| | AE† | 10 |
| Human pst | G | 20 |
| | AE | 10 |
| Rabbit pst | G | 640 |
| | AE | 160 |
| Pigeon pst | G | 160 |
| | AE | 80 |
| Human LGV | G | 20 |
| | AE | 0 |
| Human LGV | G | 40 |
| | AE | 5 |
| Human LGV | G | 320 |
| | AE | 10 |
| Human LGV | G | 20 |
| | AE | 5 |

* Group Antigen.
† Acid Extract.

to show the same specificity when assayed in infected guinea pigs. It is possible that this phenomenon may be the result of the two different animal species used to test the antigens, but a more reasonable explanation would be that a greater concentration of group antigens was affected by extraction of the virus preparations secured from allantoic fluids than from yolk sac preparations.

The fraction which lacked specificity in the allergic reaction acted as a relatively specific CF antigen. There was however a small degree of cross reactivity when the psittacosis antigen was tested with LGV antisera, and which may be due to the group antigens that were detected in the allergic phenomenon. Barwell (1952) reported that the extracts made from yolk sac preparations were not active as complement-fixing antigens, precipitinogens or when used to sensitize erythrocytes. In addition to their purer state, the viral mass obtained from allantoic fluid in our studies was exceedingly greater than the quantity of virus recovered from yolk sac preparations. Since the CF activity of the antigen was of a low order, it is conceivable that little or no activity would be found in the yolk sac extracts. Attesting to the different antigenic state of the allantoic fluid virus, all of the CF and most of the skin test activity resided in the precipitate

formed (pH 4.5) upon neutralization of the extract; whereas, the neutralized supernate was found by Barwell (1952) to contain allergenic activity.

Because of the poor yields of antigen, the use of this material as a specific CF antigen is at the present time prohibitive. Only sufficient antigen for a single antigen titration and serum titration was produced from virus processed from approximately 25 dozen embryos. It was considered therefore that other methods of extraction should be attempted, and a subsequent report will describe an additional procedure of solubilizing the psittacosis-LGV antigens.

*Summary*. 1. Skin test antigens extracted with acid from psittacosis, meningopneumonitis, and lymphogranuloma venereum viruses grown in yolk sacs were specific when tested in homologously infected rabbits. Similar antigens prepared from virus obtained from allantoic fluid were not specific when tested in guinea pigs infected with psittacosis.

2. A fraction of the psittacosis acid extract obtained from allantoic fluid virus preparations showed a high degree of complement-fixing specificity.

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# THE PAIRING OF SOMATIC CHROMOSOMES: A SURVEY

JEFFREY M. N. BOSS*

Our knowledge of the reduction in chromosome number, which is an essential feature of the formation of germ cells, dates from van Beneden's observation, in 1883, that, in the equine roundworm *Ascaris Megalocephala*, the number of chromosomes in a gamete was half that in the somatic cells. The process of gametogenic reduction division (meiosis) was described in detail by von Henking (1891) and Rückert (1891), who worked with the testes of insects and the oöcytes of elasmobranch fishes respectively. Von Henking found the pairing of chromosomes to be part of the process of reduction division, and, since that time, such pairing has been recognized as an essential feature of meiosis. During the past half-century, however, a considerable number of examples of the pairing of chromosomes in somatic cells has been reported, although pairing is not a constant feature of mitosis. Indeed, even before the existence of meiosis had been demonstrated, or even postulated (Weissmann, 1887), it had been suspected that chromosomes might pair during somatic cell division, for, 1882, Flemming wrote: "Die früher angenommene Längverschmelzung von je zwei schleifen in den Tochtersternform habe ich nach eigenen längeren Zweifeln, besonders aber jetzt auf Grund der Arbeit von Retzius aufgegeben"—"Having long had doubts, I have now given up the previously assumed longways fusion of each two loops (chromosomes) in the daughter aster (anaphase chromosome group), especially in view of the work of Retzius." As will be discussed below, chromosome pairing in anaphase, such as Flemming suspected, has been observed since his time, and, in some species, there is an apparent longways fusion.

The present communication is by no means a complete re-

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view of the publications dealing with the pairing of chromosomes in somatic cells, but is meant to indicate generally the extent of the work done on this subject.

*Diploid plants.* Among plants, the pairing of homologous chromosomes in mitosis is fairly common. Müller (1909) reported chromosome pairing in dividing cells in the root-tip of *Yucca*, and his drawings show some proximity at metaphase of chromosomes of similar form. Kuwada (1910) and Tahara (1910) reported somatic chromosome pairing in rice (*Oryza sativa*) and the mulberry (*Morus indica* L.) respectively, and Gates (1912) found two division figures with paired chromosomes in the nucellus of *Oenothera lata*, a species of evening primrose. Watkins (1935) found homologous chromosome pairing in both the root-tip and the embryo of *Yucca rupicola*. Watkin's paper contains a full review of the then published examples of somatic chromosome pairing, together with a table of 38 references to published descriptions of somatic pairing in 33 species of plant, 16 of them spermatophytes both monocotyledons and dicotyledons.

*Diploid insects.* Known examples of somatic chromosome pairing are even more abundant in dipteran insects than among plants. Stevens (1908), in a study in which he also established that the chromosome sets of the fruit-fly *Drosophila* were sexually dimorphic, found in that insect, pairing of chromosomes in all oögonia and spermatogonia, in ovarian follicle cells and in some embryonic cells. Stevens pointed out that, because of this pairing, spermatogonial mitosis could closely resemble the meiotic prophase of primary spermatocytes.

Metz (1914), in establishing that each chromosome in a complement has a characteristic and invariable form, found that, in *Drosophila*, chromosome pairing could be seen in every mitotic prophase and metaphase, but the arrangement of the chromosomes in anaphase did not permit this phenomenon to be confirmed or excluded; only the sex chromosomes sometimes failed to pair. Metz (1916) continued these studies on the diptera, finding chromosome pairing in all the mitotic diploid cells examined in 80 species. The pairing was now found to be most clearly visible in prophase and anaphase, being slightly less obvious in metaphase, while telophase and interphase were unsuitable for study.

Wherever the form of the chromosomes could be made out distinctly, pairing was seen to be homologous; the pairing was well established in the embryo at the later cleavage divisions and all subsequent stages. Whiting (1917) found pairing of chromosomes in the anaphase of mitosis in the mosquito *Culex pipiens* L.

Metz and Nonidez (1921) described how, in the fly *Asilus sericeus* Say, chromosome pairing in the last spermatogonial anaphase is followed by a meiotic prophase in which the chromosomes are already paired. There is no leptotene stage, although the nuclear chromatin is diffuse in appearance during the interphase between the mitotic and meiotic divisions.

Robertson (1930) found somatic chromosomes paired in partheno-produced diploid insects of the orthopteran species *Apotettix eurycephalus* and *Paratettix texanus*.

*Other organisms.* The only published example of the pairing of chromosomes in somatic cells of animals other than insects is that described by Boss (1953, 1954), who found that, in cultures of tissues of the Adriatic crested newt *Triturus cristatus carnifex*, the number of chromosomes in dividing fibroblasts appeared to become halved during anaphase. This finding he confirmed on fixed and stained tail-tips of hybrid *Triturus* tadpoles which varied in ploidy from one to four. In any one tail-tip, whatever its ploidy, the apparent number of chromosomes in a single anaphase group was half that of the chromosomes in a metaphase plate. Serial photographs of living fibroblasts in cultures of newt tissues showed chromosomes in the process of pairing, the members of a pair approximating side by side until they were so closely apposed that they appeared to have fused into a single body. The members of a pair could be separated partially, by squashing in 45 per cent acetic acid, and completely, by treating the living cells with hypotonic saline before fixation. That pairing may take place in anaphase had previously been noted by Whiting (1917) and Grell (1946) in the mosquito *Culex*, while the closeness with which chromosomes could pair in somatic cells was noted by Metz (1914) who wrote that "the members of each pair become so intimately united that they may be said actually to conjugate."

Hsu (1954) has found that similar chromosomes may be seen

lying parallel in pairs in the cells of mammalian tissues cultivated *in vitro*, after considerable disruption of the chromosome figure by hypotonic saline.

*Interpretation of somatic chromosome pairing.* Since, as in Boss's studies, pairing may cause two chromosomes to appear as one and so cause an apparent halving of the chromosome number, a new interpretation is possible of findings such as those of Lindahl (1953) and Kinosita *et al.* (1954). Lindahl found that, in the "small micromeres" of the sea urchin embryo, anaphase groups may have the haploid number of chromosomes; he saw secondary chromosome pairing at metaphase and concluded that reduction division occurred. Kinosita *et al.* noted an apparent reduction in chromosome number in normal myelocytes of the Japanese albino rat, but the "haploid" cells of which they write may be diploid cells with close pairing of chromosomes.

Another consequence of very intimate pairing is that re-separation of the members of a pair may be mistaken for the splitting of a chromosome. For example, the apparent splitting of telophase chromosomes in the frog (Kater, 1927), the rat (Kater, 1928), the axolotl *Amblystoma tigrinum* (Dearing, 1934) and flowering plants (Koerperich, 1930), might be the separation of chromosomes that had previously paired. A similar explanation could account for the double structure of anaphase chromosomes in *Amblystoma punctatum*, reported by Creighton (1937).

*Homologous chromosome groups larger than pairs.* It is not only in the diploid state that homologous chromosomes may come together in mitosis. Metz (1916), after studying 80 species of diptera, stated that the pairs of chromosomes in diploid insects were replaced in polyploid individuals by groups containing homologous chromosomes corresponding in number to the ploidy. In the alimentary tract of the mosquito *Culex pipiens* cells may, during metamorphosis, increase in chromosome number up to 64-ploid, and Holt (1917) found that sister chromosomes are grouped together in mitosis, the number in a group corresponding to the ploidy. Grell (1946), in a study of the chromosomes in the ileum of this insect, found that homologous chromosomes come together in anaphase, and that the chromosomal reduplication which leads to polyploidy in the organ tends to inhibit active pairing.

Ishikawa (1911) found somatic pairing in examples of *Dahlia coronata* which had a tetraploid chromosome number, but he does not mention groups larger than pairs. Similar appearances are recorded by Berger and Witkus (1948), who produced tetraploid mitotic figures in root-tips of the onion *Allium* by using alpha-naphthalene acetic acid, but it is not certain whether the added substance caused the tetraploidy or whether it brought into mitosis existing tetraploid cells; in prophase and early metaphase chromosomes were arranged in a diploid number of pairs. Ruys (1924, 1925) has, however, found in polyploid plants groups of chromosomes corresponding in number to the ploidy.

*Secondary chromosome pairing in meiosis.* Chromosome pairing in somatic cells may, in certain respects, be comparable with secondary pairing in meiosis. This secondary association, unlike primary pairing, does not occur until just before the first meiotic metaphase, is not accompanied by the formation of chiasmata, and may persist until second meiotic anaphase. Lawrence (1931) has described secondary pairing in *Dahlia*, and his paper reviews a number of examples of the secondary association of bivalents in meiosis.

*Induction of pairing.* Creech (1942) added 2:5:6 dibenzanthracene choleic acid to cultures of chick fibroblasts and found loops in prophase chromosomes. She interpreted this as premature chromatid separation, but the loops, which are well seen in the published photographs, may have been spaces between paired chromosomes.

*The question of homology.* Metz (1916), in his survey of somatic chromosome pairing in over 80 species of dipteran insect, found that, whenever the form of individual chromosomes was distinguishable, they were arranged in homologous pairs. Pairing between non-homologous chromosomes must have occurred, however, in the tail-tips of hybrid *Triturus* tadpoles examined by Boss (1953, 1954), for an apparent halving of the chromosome number in anaphase groups was found even in haploid individuals, and, as has been pointed out, this apparent diminution in number is, in *Triturus*, attributable to the pairing of chromosomes. In view of Boss's finding, it may be necessary to regard the attractive forces in somatic chromosome pairing as able to act with less specificity than has been assumed by, *e.g.*,

Fabergé (1942) in his discussion of the underlying mechanism of homologous chromosome pairing.

*Chiasmata.* Darlington (1930), from observations on the fritillary *Fritillaria imperialis*, concluded that primary pairing in meiosis depends upon chiasma formation. It is certain that chiasmata are not a necessary feature of somatic chromosome pairing, but some authors have reported their presence in somatic cells. Huskins (1948), for example, mentions chiasmata in onion roots.

Farmer, Moore, and Walker (1903 a and b) described chromosomal loops and rings in human epitheliomata, and found that the number of such bodies in a cell was half the expected number of chromosomes. Ewing (1940), citing the work of Farmer *et al.*, describes such "gametoid" divisions as a normal feature of malignant growths. Bashford and Murray (1906) reported a similar finding in a carcinoma of mice; these authors claimed that von Hansemann, in 1893, saw reduction divisions in malignant tumours, but interpreted what he saw as the result of assymetrical mitosis, chromosome degeneration and the clumping of chromosomes. Von Hansemann's own interpretation may have been correct, of course. The illustrations to the papers by Bashford and Murray show chromosomes which may or may not be bivalents similar to those of meiotic diakinesis.

Other workers have found that physical or chemical interference with tissues might lead to chiasma formation. Peto (1935) found that, in barley *(Hordeum vulgare)* kept at 35°–36° C., chromatid breaks with misunion could be found within single chromosomes. Hearne (1936) induced chiasma formation in mouse fibroblasts by adding to tissue cultures methylcholanthrene in a concentration of 0.005 mgm./cc.; the frequency of chiasmata corresponded to that in normal meiosis.

If, as is generally accepted, there is an association between structural chiasmata and genetic crossing-over, then the latter may indicate the former. Muller (1916) inferred genetically the occurrence of somatic crossing-over in a mosaic *Drosophila*, and similar conclusions have been drawn by Stern (1935, 1936) from mosaics of the same genus, the latter author, in 1939, defending his view that homologous interchanges occur in somatic cells. Bateson (1926), reviewing genetical segregation, has discussed its occurrence in somatic cells.

*Reduction division.* Primary pairing in meiosis is preparatory to reduction division, but somatic chromosome pairing is generally followed by an equational mitosis. However, there are a number of reported examples of reductional division in somatic tissues, and some of these are in species in which somatic chromosome pairing is known to occur, *e.g.*, the mosquito *Culex pipiens* (Berger, 1938). A full discussion of reduction in chromosome number in somatic tissues is outside the scope of this review.

## *Conclusions*

1. Homologous chromosomes of somatic cells are known to pair in certain species of plants, insects and amphibia.

2. Pairing may perhaps sometimes take place between non-homologous chromosomes.

3. When chromosomes associate so intimately that two appear as one, there may be an apparent reduction in chromosome number, and re-separation within a pair is liable to be misinterpreted as the splitting of a single chromosome.

4. Somatic pairing may occasionally be associated with chiasma-like unions between chromosomes, and, in other examples, with subsequent reduction division.

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# THE EFFECT OF DEMEROL AND MORPHINE ON THE HISTOLOGIC STRUCTURE OF MOUSE TISSUES

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The histological effects of morphine derivatives may well be considered in relation to their general pharamacological actions.

*Demerol.* Intravenous injections of demerol generally lowers the blood pressure by vasodilation, even when relatively small doses are given (Gruber *et al.*, 1941). It resembles morphine in its action as a respiratory depressant, and in slowing the heart. Demerol acts as an analgetic and spasmolytic drug, causing a relaxation of the smooth muscles of the blood vessels and body. The relaxation of the smooth muscles of the blood vessels causes a peripheral vasodilation and thus lowers blood pressure. The drug exerts this same spasmolytic action on any viscus containing smooth muscle, and the action is greater if the muscle is contracting (Yonkman, 1943). It has little effect on the intestine (Sollmann, 1948).

Mice respond to toxic doses of demerol by increased general activity, clonic convulsions, Straub reaction, marked cyanosis and often death due to respiratory failure (Gruber, Hart, and Gruber, 1941). Way, Gimble, *et al.* (1949) demonstrated that after the administration of demerol to rats, the compound concentrates mainly in the kidneys, spleen, lungs and liver. Appreciable levels are attained in the brain, and lower concentrations in the heart and muscle. After repeated injections of demerol three times daily for several days, virtually no demerol could be recovered from the animals 24 hours later. The rapid inactivation of demerol lessens the possibility of the occurrence of cumulative effects.

*Morphine.* White mice respond to the hypodermic injection of morphine (0.02 mg.) or its esters by characteristic S-shaped stiff erection of the tail. This is the Straub reaction. The morphine alkaloids may produce catatonic rigidity, acting probably through the midbrain. In rats the rigidity is plastic, so that the animals tend to preserve imposed awkward positions.

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The cerebral effects are almost purely depressant (analgesia, lethargy, stupor, sleep and coma) in the dog, rabbit, guinea pig, rat, mouse and also in birds. Ordinarily the circulation remains fairly good, until death occurs by paralysis of respiration (Sollmann, 1948).

With intraveneous injections of morphine, only traces remain after 2 to 10 minutes in dogs (Hatcher and Gold, 1929). In general the largest quantity is in the skeletal muscles; then the kidneys, liver, and intestinal contents; the brain contains very little, less than the blood, even in a very severe poisoning (Hatcher and Gold, 1929; P. Fleischmann, 1931).

In studies with radioactivity March and Elliott (1952) demonstrated that both sexes of rats eliminate virtually all radioactive morphine within 48 hours; about ⅔ via the urine, and ⅓ via the gastrointestinal tract. In the central nervous system they found virtually no radioactivity but they did find significant amounts in the lungs and spleen at one hour. By six hours, however, most of the radioactivity was in the urine or gastrointestinal tract.

The liver plays an important part in the destruction of morphine; liver damage increased excretion by an average of 43 per cent in habituated and nonhabituated animals (Gross, Plant, and Thompson, 1938). On perfusion through the liver, morphine is rapidly fixed and destroyed, the rate being the same in habituated and nonhabituated animals (Hatcher and Gold, 1929).

With toxic doses, fall in blood pressure is more marked (central vasomotor depression) and the rate of the pulse varies. Stimulation of the vagus center and disturbed cardiac conduction often causes arrhythmic and weak pulse. The heart muscle suffers indirectly by the lower blood pressure and asphyxia (hence the danger of cardiac insufficiency, especially when combined with dyspnea). Ordinarily the circulation remains fairly good, until death occurs by paralysis of respiration (Sollmann, 1948). Morphine affects the respiratory center centrally, thus causing anoxia, which, if it lasts long enough will cause hyperemia, edema, and hemorrhage. This is the general tissue response to anoxia irrespective of cause.

*N-Allynormorphine.* If n-allynormorphine is given before or after the administration of morphine, it prevents the respiratory

depression caused by the morphine (Tullar, 1951). Hart and McCawley (1944) and Unna (1943) investigated the ability of n-allynormorphine to prevent respiratory failure caused by normally lethal doses of morphine. These authors demonstrated that the respiratory depressant action of morphine is antagonistic to n-allynormorphine. Huggins, Glass, and Bryan (1951) showed that the protective action of n-allynormorphine extended to several narcotics, but not to demerol. They demonstrated that n-allynormorphine does not prevent the lowering of blood pressure caused by morphine.

Radoff and Huggins (1951) found that allynormorphine does have the ability to protect against a lethal dose of demerol and that this may be due to antagonism of the respiratory depression caused by demerol. They indicate that n-allynormorphine must be injected within one minute after demerol to obtain protection, which is evidently because of the rapidity of action of demerol. They also note occasional convulsions after the injection of demerol, thus demonstrating the rapidity of action of this drug.

Moller (1954) stresses the fact that the treatment of morphine poisoning should consist of artificial ventilation of the lungs with oxygen, in addition to the injection of n-allynormorphine. If the respiratory aid is omitted, anoxia will often occur, which leads to secondary circulatory shock.

The results of the study on drug toxicity by Pomerat, Drager, and Painter (1946) suggest that the study should be extended to include particular tissues, in addition to gross lethal effects. In view of the fact that many drugs do accumulate rapidly in the organs following injections, it was decided that the present study was indicated in an effort to determine histological changes which might occur.

*Materials and Methods.* The animals for the present study were furnished by a colleague, S. E. Huggins, who was determining the LD 50 of several drugs for a given population of mice. Her experiments involved the extent of the protection of demerol against n-demerol, morphine against demerol, as well as other drug combinations as presented in Table 1. She used 26 experimental mice of the Harlan Swiss albino strain and divided them into eight groups. These animals, all mature males, were injected subcutaneously with the drugs, which were prepared in distilled

TABLE 1

| Group No. | No. Animals in Group | Drug Dosage | Aver. Survival Time (minutes) |
|---|---|---|---|
| I | 4 | 200 mg./kg. demerol | 36.5 |
| II | 4 | 190 mg./kg. demerol plus 20 mg./kg. n-demerol 1 min. later | 37.5 |
| III | 2 | 190 mg./kg. demerol 40 mg./kg. n-demerol 1 min. later | 13.5 |
| IV | 2 | 400 mg./kg. n-alloy-nordemerol | *170<br>*1080 |
| V | 4 | 1000 mg./kg. n-allynormorphine | *260<br>†100 |
| VI | 4 | 500 mg./kg. morphine | 115.7 |
| VII | 4 | 475 mg./kg. morphine 40 mg./kg. demerol 15 min. later | 70.2 |
| VIII | 2 | 475 mg./kg. morphine 40 mg./kg. n-demerol 15 min. later | 54 |
| IX | 6 | ..... | .... |

* Mouse sacrificed by severing spinal cord.
† Average for 3 mice; 4th was sacrificed.

water according to standard procedures. Six control mice, as well as three experimental mice which survived the injections were sacrificed by the rapid method of severing the spinal cord. All of the mice which were used in the present investigation were received and fixed immediately after death.

Table 1 demonstrates the eight groups of animals, the drug dosage, and the survival time in minutes following the injections. All animals which died following the injections of the drugs were immediately autopsied and their organs placed in fixatives. Brain and spinal cord were fixed in absolute alcohol. Ten per cent formalin neutralized with calcium chloride was used to fix the heart, lungs, kidneys, spleen, pancreas, liver, and testis. Baker (1945) recommends the use of calcium chloride with formalin to counteract the gross cell shrinkage produced by diluted formaldehyde. Paraffin sections were cut 6 to 10 micra thick prior to staining. Harris' hematoxylin stain with eosin counterstain was used routinely. Mallory's fuscin stain was used to demonstrate hemosiderin and other hematogenous pigments (Mallory, 1938).

## *Results*

Group I. In this group were four mice which received 200

mg./kg. demerol. The average survival time was 36.5 minutes. Examination of the liver and kidneys showed petechial hemorrhages in these organs of three mice. Both kidneys were affected in each of the three animals. The use of Mallory's fuscin stain demonstrated that the epithelial cells of the liver and kidneys contained abnormal quantities of hemosiderin. The pigment was the result of hemorrhages or of general hemolysis. The hemosiderin appeared as bright yellowish brown granules and masess in paraffin sections. Using the Mallory's technique it was not pos-

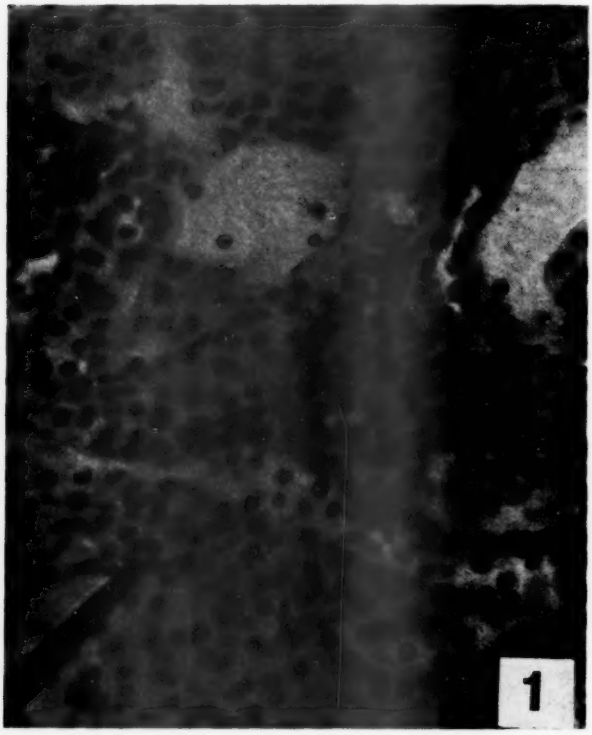


PLATE 1. Kidney of male mouse which was injected subcutaneously with 200 mg./kg. demerol. This animal lived 25 min. Hemorrhagic areas are seen in the upper left and lower right-hand corners. Hematoxylin-eosin stain. X approximately 860.

sible to demonstrate hematoidin in the sections, indicating that the hemorrhages were not old ones. Hematoidin may assume the form of brown rhombic crystals or of amorphous granules seen in the neighborhood of any old hemorrhage (Boyd, 1953). Plate 1 demonstrates the petechial hemorrhages in the kidney, and plate 2 shows the same lesion in the liver.

A consistent abnormality seen in the kidneys of those animals in group I was the distension of the veins. Also noted in all four mice was an increased space between the glomerular tuft and the Bowman's capsule, but this perhaps was due to fixation.

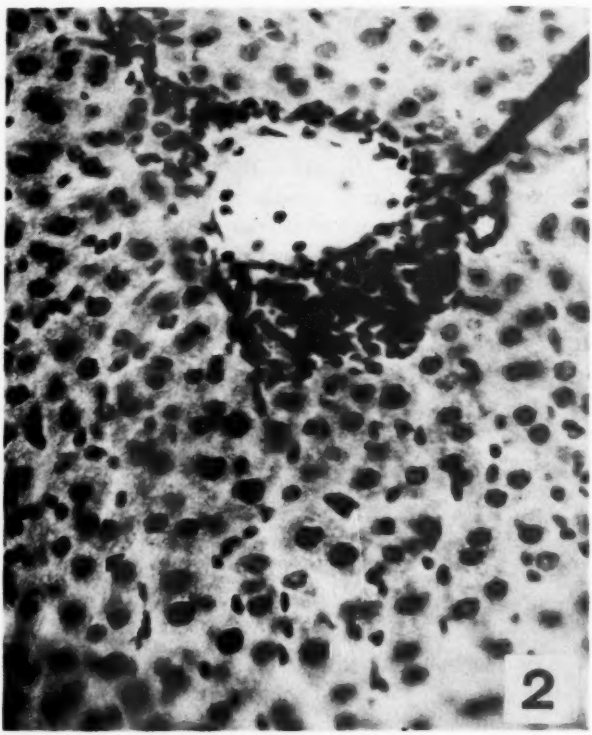


PLATE 2. Liver of male mouse which received 200 mg./kg. demerol subcutaneously and lived 34 minutes. The petchial hemorrhage is seen surrounding a central vein and extending into the sinusoids. Hematoxylin-eosin stain. X 860.

Passive congestion of the lungs was noted, as well as thickened alveolar walls. Boyd (1953) indicates that in passive congestion the dilation of the vessels is a passive affair as a result of mechanical causes. This type of congestion occurs when the increased amount of blood in the tissue is due to venous obstruction (Anderson, 1946). Thus the blood tends to accumulate in the tissue. The walls of the alveoli in the mice of group I were often so thickened as to separate the vascular endothelium from the air in the alveoli. Plates 3 and 4 demonstrate this abnormality.

Groups II and III. The mice receiving demerol and varying amounts of n-demerol one minute later had an average survival time of 37.5 min. for group II, and 13.5 min. for group III. The only lesions in both these groups of animals were those affecting the lungs. In group II there was an inflammatory cell infiltration in the tissue which is suggestive of pneumonitis. The capillaries were congested and the alveoli somewhat edematous, although this condition was more obvious in the gross specimen, and was seen microscopically in only two mice. One animal of group III showed a moderate amount of edema in the lungs which were examined grossly, but this condition was not corroborated microscopically.

Group IV. No pathological changes were seen in the organs of the mice in group IV. These animals received 400 mg./kg. n-allynordemerol but both were sacrificed, one after 2 hrs. and 50 min., and one after 18 hours.

Groups V, VI, VII, and VIII. The livers of the animals in these four groups were congested with blood, apparently as a result of the anoxia. These mice received varying doses of n-ally-normorphine, morphine, morphine plus demerol, and morphine plus n-demerol, as noted in table 1. The mice in these four groups had very compressed liver cords and wide open sinuses. An unusual number of white blood cells had infiltrated into the sinusoids, and the periportal areas contained an increased number of leucocytes over the normal condition. Small areas of hemorrhage were seen in 3 of the mice which received only morphine. These are demonstrated in plate 5. Groups VI, VII, and VIII showed congestion of the lungs with blood, and this condition of hyperemia was most pronounced in those animals of group VI which lived an average of 115.7 min. after the injection of 500 mg./kg. morphine. The

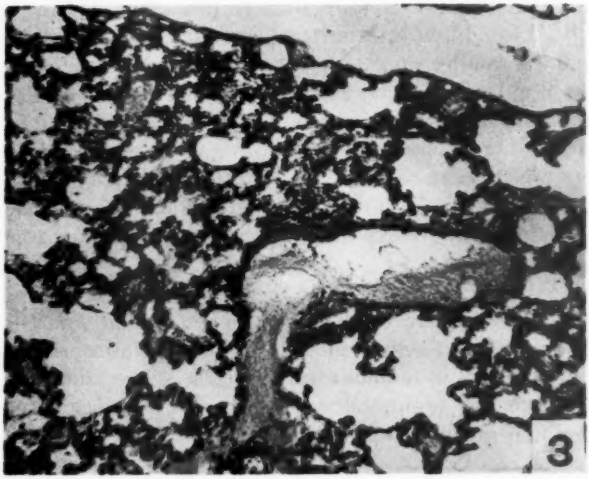


PLATE 3. Lung of mouse which received 200 mg./kg. demerol and died 25 min. later. Thickening of the alveolar walls is seen here. Hematoxylin-eosin stain. X 860.

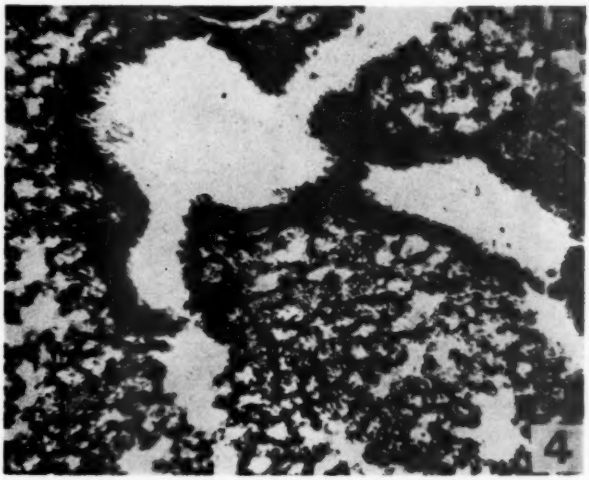


PLATE 4. Lung of mouse which received 200 mg./kg. demerol and which lived 34 min. after the injection. Thickening of the alveolar walls is most pronounced in the region of the bronchus. Hematoxylin-eosin stain. X 860.

lungs of these animals were slightly edematous, as examined grossly. A condition of arteritis in the lungs was more obvious around the smaller vessels than the larger ones (Plate 6).

The number of macrophages seen in the spleens of all mice were within the normal range, and no lesions were noted in this organ. Similarly, no abnormalities were demonstrated in the hearts, pancreas, testis, brain, or cord. The lack of abnormalities in the nervous system is difficult to understand, inasmuch as the nerve tissue would be expected to show the effects of anoxia most clearly.

Anderson (1946) states that the post mortem on morphine-poisoned subjects reveals no significant changes, and Boyd (1953) indicates likewise that there are no characteristic post-mortem changes in human beings killed with the drug. The evidence from this preliminary work therefore leads to the conclusion that further investigation of the histologic picture should be attempted.

*Summary*. Demerol, a respiratory depressant, when injected into mice in lethal doses, causes death apparently as a result

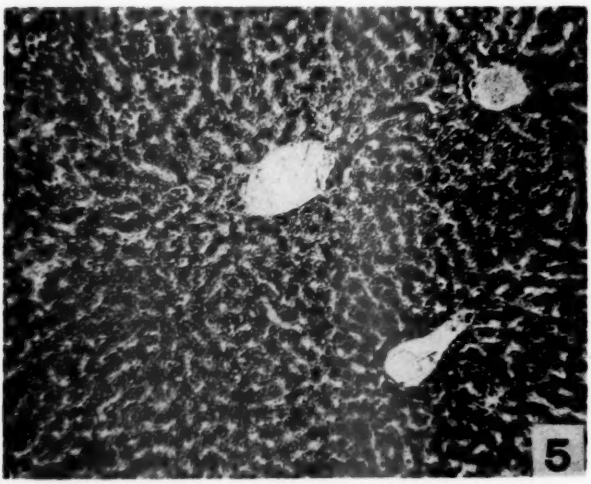


PLATE 5. Liver of mouse which received 500 mg./kg. morphine, and lived 49 min. after the injection. Small hemorrhages are seen in the upper left-hand corner of the photomicrograph. Hematoxylin-eosin. X 860.

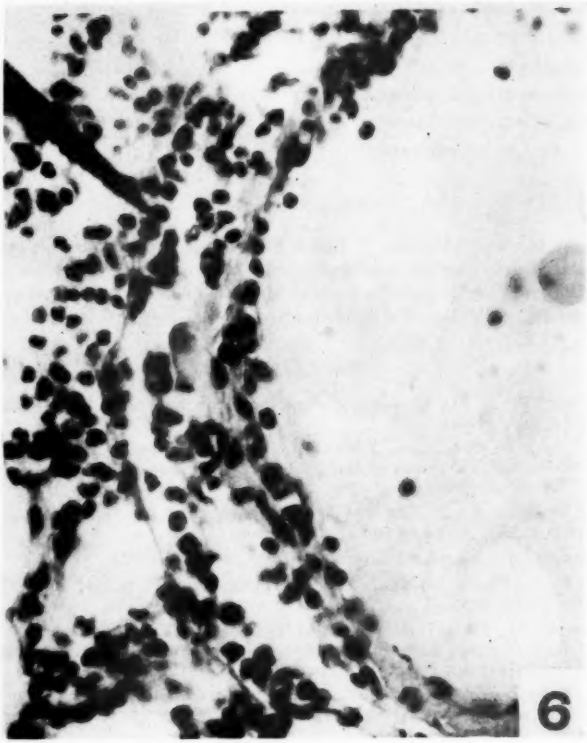


PLATE 6. Lung of mouse which received 500 mg./kg. morphine and which lived 120 min. Hematoxylin and eosin stain used to demonstrate arteritis. X 860.

of respiratory failure. Microscopic examination reveals petechial hemorrhages in the kidney and liver and distension of the veins of the former organ. Passive congestion occurs in the lungs.

Demerol, plus n-demerol affects only the histologic structure of the lungs, which are edematous, have congested capillaries, and exhibit a condition resembling pneumonitis.

N-allynordemerol, injected in sub-lethal amounts, causes no pathologic tissue changes.

Injection of n-allynormorphine, morphine, morphine plus demerol, or n-demerol causes very compressed liver cords, infil-

tration of leucocytes into sinusoids, and hyperemia in lungs. The latter condition is most pronounced after the injection of morphine alone.

In view of the prevailing opinion that these drugs cause no characteristic histologic alterations in tissues, further investigation seems to be indicated.

*Acknowledgments*

I wish to thank Mr. John T. Ferris of the University of Houston Photography department for making the excellent photomicrographs.

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# REPORT ON THE USE OF PHENOLIZED RABIES VACCINE IN TEXAS FROM 1949 THROUGH 1953

E. B. M. Cook, Caroline Stearns, Julian Feild, and J. V. Irons*

The use of vaccine for the emergency treatment of rabies is widely recommended by public health authorities largely because of the remarkably low mortality rate among vaccine-treated persons. McKendrick (1940) in his ninth review cited an overall mortality rate from rabies in treated persons of 0.33 per cent. Physicians and health officers in highly endemic areas unfortunately are confronted with the management of both real and imaginary exposures. Those who have imaginary exposure and suffer from "rabiphobia" (Sellers, 1948) constitute a formidable group which certainly can disturb the physician's professional equilibrium. It will be shown that the complications of antirabic vaccination are sufficiently common that this treatment is not recommended for those who suffer from "rabiphobia" in the absence of a wound by the teeth of a rabid animal.

Since 1934, a modified Semple, phenol-treated rabies vaccine has been prepared and distributed throughout the state by the Texas State Department of Health. This vaccine is a brain tissue suspension from rabbits infected with a "fixed strain" of rabies virus. The virus is killed by the addition of 1.0 per cent phenol to a 20 per cent brain suspension. After the virus is killed, the vaccine is adjusted to a final concentration of 5.0 per cent brain tissue and 0.25 per cent phenol. It is preserved with merthiolate in a 1:10,000 concentration. The potency of the vaccine is determined by a mouse protection test and compared with that of a standard Reference vaccine preparation provided by the licensing agency of the National Institutes of Health. The finished vaccine must provide at least the same degree of protection as the Reference Rabies vaccine and must pass the requisite tests for sterility and animal safety.

The decision to give or to withhold antirabic vaccine treatment following exposure to a rabid animal is left entirely to the attend-

* From the Biologics Division, Bureau of Laboratories, Texas State Department of Health, Austin, Texas. Received for publication February 5, 1955.

ing physician. The Texas State Department of Health makes certain recommendations among which are:

1. All wounds inflicted by animals should be washed immediately and thoroughly for 15 or 20 minutes with a warm strong soap solution.

2. A positive diagnosis of rabies, confirmed by laboratory test whenever it is possible, should be made by a veterinarian before rabies vaccine is administered.

3. Antirabic vaccination is indicated for all direct exposures, that is, teeth wounds made by rabid animals.

A. The vaccine should not be given merely because of minor cuts or abrasions in the absence of a bite; minor abrasions or bruises made by the teeth over the clothing do not indicate the need of antirabic treatment.

B. Scratches made by the animal's claws do not indicate need of antirabic treatment.

4. In case of severe bites about the head or face, or severe multiple lacerations, the vaccine treatment should be intensified by increasing the total dose to 21 or 28 doses, given two a day.

A. If prophylactic antirabies serum is available it should be given without delay.

5. When potentially serious systemic reactions occur, the use of the vaccine should be discontinued, unless risk of infection is great.

A. "Treatment paralysis" is a definite hazard.

Desiccated cord vaccine was dispensed in Austin, Texas, from 1904 until 1934 when state-wide distribution of the phenolyzed vaccine was begun. The sporadic reports of reaction to the vaccine and of treatment failure had long suggested the need for collection of data on the use of the rabies vaccine. Beginning in 1949, each 14-dose vaccine treatment was accompanied by a report blank with a request for information as to the diagnosis of the animal, nature and location of the bite, date of bite and starting treatment, the number of doses of vaccine and how injected, and reaction to treatment. During the five-year period, 15,201 rabies vaccine treatments were prepared and distributed by the State Health Department. Of these, 1,276 treatments were returned unused. Complete reports were received on the use of 8,430 treatments (Table 1). There were no deaths from rabies in treated persons in this series, although we had learned of one treatment failure in 1946 and another in 1948.

During the 1949–1953 period, 6,383 or 28.2 per cent of the 22,632 animal heads examined by the State Department of Health were found to be rabid. This data is presented in table 2. Since most rabid wild-life animals presumably are not subjected

to laboratory test, it is obvious that rabies was and is quite prevalent in Texas.

In the questionnaires twenty-three species of offending animals were mentioned, with laboratory proof of rabies cited for sixteen species. This data is presented in table 3. Despite the ap-

TABLE 1

*Use of Rabies Vaccine in Texas for the Years 1949 Through 1953*

| Lot Number Vaccine | Number Treatments Distributed | Number Treatments Not Used, Out Date, Etc. | Number Reports On Use |
|---|---|---|---|
| R 223 | 532 | .. | 191 |
| R 224 | 419 | .. | 249 |
| R 225 | 437 | .. | 387 |
| R 226 | 514 | .. | 223 |
| R 227 | 472 | .. | 197 |
| R 228 | 357 | .. | 111 |
| R 229 | 200 | .. | 53 |
| R 234 | 422 | .. | 199 |
| R 235 | 460 | .. | 264 |
| R 239 | 518 | .. | 295 |
| R 240 | 435 | 2 | 193 |
| R 241 | 481 | 1 | 332 |
| R 242 | 506 | 8 | 381 |
| R 243 | 494 | 15 | 249 |
| R 244 | 498 | 128 | 218 |
| R 245 | 487 | 200 | 223 |
| R 246 | 444 | 33 | 311 |
| R 247 | 361 | 32 | 221 |
| R 248 | 365 | 69 | 218 |
| R 249 | 459 | 137 | 260 |
| R 250 | 348 | 43 | 176 |
| R 251 | 417 | 54 | 250 |
| R 252 | 301 | 56 | 126 |
| R 253 | 364 | 41 | 245 |
| R 254 | 397 | 65 | 244 |
| R 255 | 126 | 1 | 125 |
| R 256 | 454 | 71 | 268 |
| R 258 | 334 | 52 | 254 |
| R 259 | 478 | 41 | 289 |
| R 260 | 224 | .. | 179 |
| R 261 | 361 | .. | 108 |
| R 262 | 272 | 30 | 184 |
| R 263 | 154 | 30 | 68 |
| R 265 | 254 | 34 | 161 |
| R 266 | 282 | 22 | 167 |
| R 267 | 287 | 21 | 157 |
| R 268 | 243 | 51 | 149 |
| R 269 | 253 | 33 | 144 |
| R 270 | 318½ | .. | 199* |
| R 271 | 412 | 6 | 162* |
| | 15,201½ | 1,276 | 8,430 |

* Reports on use not complete at time of compiling data.

parent increased prevalence of rabies in wild-life, notably the fox and skunk, it was of interest to note that the dog and cat were far more frequently the offending animal in the exposure of humans to rabies (Sullivan *et al.*, 1954 and Irons *et al.*, 1954).

TABLE 2

*Data on Rabies in Texas From 1939 Through 1953*

| Year | Number Cases Human Rabies | Number Treatments Rabies Vaccine Distributed | Animal Rabies | | |
|---|---|---|---|---|---|
| | | | Number Heads Examined | Number Positive | Per Cent Positive |
| 1939 | 1 | 1,543 | 2,038 | 583 | 28.56 |
| 1940 | 1 | 1,648 | 1,528 | 394 | 25.79 |
| 1941 | 5 | 1,539 | 1,661 | 347 | 20.89 |
| 1942 | 1 | 1,564 | 1,742 | 497 | 28.53 |
| 1943 | 5 | 1,871 | 2,126 | 788 | 37.06 |
| 1944 | 4 | 1,649 | 2,960 | 910 | 30.74 |
| 1945 | 2 | 1,594 | 2,742 | 838 | 30.56 |
| 1946 | 6 | 2,220 | 3,619 | 1,245 | 34.40 |
| 1947 | 1 | 2,475 | 3,913 | 1,099 | 28.09 |
| 1948 | 5 | 3,512 | 4,787 | 1,248 | 26.82 |
| 1949 | 0 | 2,978 | 4,113 | 1,000 | 24.31 |
| 1950 | 1 | 2,999 | 4,542 | 1,220 | 26.86 |
| 1951 | 3(1)* | 3,487 | 4,635 | 1,373 | 29.63 |
| 1952 | 6 | 3,191 | 4,987 | 1,462 | 29.31 |
| 1953 | 1 | 2,556 | 4,355 | 1,328 | 30.49 |
| Sub Total | 11(12)* | 15,201 | 22,632 | 6,383 | 28.20 |
| Total | 42(43)* | 34,826 | 49,748 | 14,368 | 28.88 |

* Bat rabies case.

Forty-two human deaths from rabies in Texas were recorded by the Bureau of Vital Statistics during 1939 through 1953. Several of these cases were not confirmed by laboratory tests. As far as could be determined only two of the 42 reported cases during this fifteen-year period had received antirabic vaccine.

From the data presented in table 4 we see that twelve deaths from rabies were recorded during 1949 through 1953 in untreated persons. The Negri body test was positive in ten cases and animal inoculation was positive in the three cases tested. Five of the cases were known to have suffered bites from stray dogs, only one of which was diagnosed as a rabid animal. The source of infection was undetermined in five cases. One of the twelve deaths was a 43-year-old female who died of a massive pulmonary embolism associated with an encephalitic condition four or five weeks after being bitten by a sick bat. Post-mortem findings on this patient revealed "demonstrable Negri bodies in the central

motor neurons compatible with rabies" (Sulkin and Greve, 1954).

TABLE 3

*Use of Rabies Vaccine Following Exposure to Various Animals and Man During the Years 1949 Through 1953*

| Animal | Total | Brain Examined | | Veterinary Diagnosed Rabies | Brain not examined because animal was | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Positive | Negative | | Lost | Alive | Killed or Dead | Decomposed or Unsatisfactory | Information Not Given |
| Dog | 7,320 | 4,839 | 86 | 10 | 603 | 340 | 455 | 66 | 921 |
| Cat | 433 | 167 | 7 | 1 | 81 | 9 | 85 | 28 | 55 |
| Cow | 119 | 64 | 8 | 3 | .. | .. | 36 | 1 | 7 |
| Calf | 110 | 69 | 2 | 6 | 1 | 1 | 21 | .. | 10 |
| Fox | 70 | 43 | .. | .. | 7 | .. | 13 | .. | 7 |
| Rat | 49 | .. | .. | .. | 32 | .. | 9 | .. | 8 |
| Skunk | 50 | 25 | .. | .. | 14 | .. | 10 | 1 | .. |
| Squirrel | 39 | 4 | .. | .. | 16 | .. | 12 | 3 | 4 |
| Horse | 26 | 16 | .. | .. | .. | 2 | 6 | 1 | 1 |
| Rabbit | 18 | 3 | 1 | .. | 6 | .. | 4 | .. | 4 |
| Raccoon | 18 | 12 | .. | .. | 1 | .. | 1 | 1 | 3 |
| Opossum | 8 | 2 | .. | .. | 2 | .. | 3 | 1 | .. |
| Civet cat | 7 | 5 | .. | .. | 1 | .. | .. | .. | 1 |
| Gopher | 4 | 4 | .. | .. | .. | .. | .. | .. | .. |
| Bull | 4 | .. | .. | .. | .. | .. | 4 | .. | .. |
| Wolf | 3 | 3 | .. | .. | .. | .. | .. | .. | .. |
| Goat | 2 | 2 | .. | .. | .. | .. | .. | .. | .. |
| Monkey | 6 | 4 | .. | .. | .. | 1 | .. | .. | 1 |
| Mule | 1 | .. | .. | .. | .. | .. | 1 | .. | .. |
| Deer | 1 | 1 | .. | .. | .. | .. | .. | .. | .. |
| Chipmunk | 1 | .. | .. | .. | 1 | .. | .. | .. | .. |
| Hog | 1 | .. | .. | .. | .. | .. | 1 | .. | .. |
| Mouse | 2 | .. | .. | .. | 1 | .. | 1 | .. | .. |
| Bat | 1 | .. | .. | .. | 1 | .. | .. | .. | .. |
| Sheep | 1 | 1 | .. | .. | .. | .. | .. | .. | .. |
| Weasel | 1 | .. | .. | .. | .. | .. | .. | 1 | .. |
| Man | 37 | 34 | 3 | .. | .. | .. | .. | .. | .. |
| Not given | 106 | 47 | .. | .. | 6 | .. | 3 | .. | 50 |
| Total | 8,438* | 5,345 | 107 | 20 | 773 | 353 | 665 | 103 | 1,072 |

* Four persons were exposed by two dogs each and 4 persons were exposed by a dog and cat each giving a total of 8,430 treatments of vaccine used.

It is known that a minimum of 39 persons received antirabic vaccine two to three months after being bitten by a rabid animal which was probably too late to confer any degree of protection, but there were no deaths from rabies in this group. Furthermore, it is known that in Texas a negative laboratory report is generally accepted as conclusive evidence that the animal in question was not rabid. However, there is good evidence (Leach, 1938, and Damon and Sellers, 1941) that the sensitivity of the

TABLE 4

*Reported Rabies Deaths in Texas for the Period 1949 Through 1953*

| Patient Age | Sex | Type of Exposure Animal | Exposure | Symptoms |
|---|---|---|---|---|
| 7 | M | stray dog | superficial wound by tooth or claw on left arm | First symptoms 10-20-50; first seen 11-2-50; child apprehensive and hard to approach; definite hydrophobia; rapid respiratory paralysis and death at 1:30 P.M. 11-2-50; post mortem showed brain positive for rabies by the Negri body test. |
| 29 | M | unknown | | Onset 3-26-51; pain in left arm of aching type gradually moving up arm to pectoral muscle and radiating to left scapular area; gasping respiration; apprehensive; fever 99 to 104° F., emesis; death 3-31-51; post mortem showed brain positive for rabies by Negri body test. |
| 4 | M | no history of exposure | | Death 5-31-51. Formol preserved specimen of brain tissue positive for rabies by the Negri body test. |
| 43 | F | sick bat | bite on hand | Bitten 9-25-51; on 11-1-51 became ill; aching; weakness left arm; nausea; emesis; difficulty in swallowing; increased salivation; paralysis of left arm; coma and death 11-5-51; post mortem showed the brain positive for rabies by the Negri body test. |
| 9 | F | no history of exposure | | Illness of one hospital day; death 11-2-51; Bureau of Vital Statistics death Certificate—rabies. |
| 9 | M | stray dog | bite on hand | Bitten September, 1951; hospitalized in coma 1-1-52; post mortem showed brain positive for rabies by Negri body test and animal inoculation. |
| 26 | M | stray dog | deep bite on face; wound treated | Bitten 12-18-51; on 1-7-52 noted earache; restless; excited; emesis of foamy blood-streaked material; army hospitalized; symptoms fulminant pneumonitis; death 1-31-52; post mortem showed brain positive for rabies by Negri body and animal inoculation tests. |
| 17 | F | stray dog | superficial bite on hand | Bitten August, 1951; on 4-7-52 inability to swallow; confusion; irritation; tonic and later clonic convulsions; temperature to 106° F.; death 4-14-52; post mortem showed brain positive for rabies by Negri body test. |
| 9 | M | thought to have had bite about August 1952 | | On 10-29-52 convulsions; hospitalized; given phenabarbitol; death 10-30-52; post mortem showed brain positive for rabies by Negri body test. |
| Adult | F | rabid dog | bite on left foot | Bitten 9-1-52; symptoms 11-4-52; hospitalized; headache; fever; convulsions; violence; coma; paralysis and death; post mortem showed brain positive for rabies by Negri body test. |
| Adult | M | no information | | Death 4-24-53; Death Certificate—Rabies;* brain reported positive for rabies by animal inoculation. |
| Adult | M | no history of exposure | | Illness of 5–6 days; V.A. Hospital; positive findings at autopsy—rabies death 7-28-53. |

* Personal communication from pathologist.

Negri body test is only about 90 per cent. During the present investigation it was found that 25 (or nearly 10 per cent) of a group of 268 dog brains reported negative by the Negri body test were later proved positive by the mouse inoculation test. If a similar correction is applied to the 16,249 animal brains reported negative, a potential 1,624 (or 10 per cent) would have been positive by mouse inoculation although negative by the Negri body test. If we presume one person bitten from each of these false negative animals, there would be 1,624 persons exposed to rabies and untreated. No deaths were reported from untreated persons bitten by animals on which the report had been negative for rabies. We feel that the number of unvaccinated persons bitten by rabid animals on which no laboratory test of any kind was done was undoubtedly large, because eleven of the twelve known deaths from rabies occurred in this category. It is seen in table 5 where reports on use of the vaccine were completed, that 66.9

TABLE 5

*Use of Rabies Vaccine with Regard to Type* of Exposure*
*Exposure of Persons Receiving Treatment*

| Result of Brain Examination of Animal | Number BITTEN | Per cent BITTEN | Number CONTACT | Per cent CONTACT | Number TOTAL | Per cent TOTAL |
|---|---|---|---|---|---|---|
| Positive ..... | 3,428 | 60.7 | 2,188 | 78.6 | 5,616 | 66.62 |
| Negative ..... | 75 | 1.32 | 47 | 1.68 | 122 | 1.44 |
| Not Examined .... | 1,599 | 28.32 | 310 | 11.1 | 1,909 | 22.64 |
| No Information ... | 544 | 9.63 | 239 | 8.58 | 783 | 9.28 |
| TOTAL ..... | 5,646 | 66.9 | 2,784 | 33.02 | 8,430 | ... |

* Type of exposure—BITTEN indicates that the physician listed the actual bite wound, its site and severity. CONTACT indicates that the physician merely stated "contact" or "exposed".

per cent of the persons receiving treatment were exposed by the actual bite of an animal. In this group 66.2 per cent were bitten by animals which had been diagnosed positive for rabies.

The classification of ill-effects from antirabic treatment was difficult. The overall summary of reactions to the vaccine is presented in table 6. Local reactions were recorded with surprising frequency, usually marked "slight" or "moderate" (Table 7). There were in all 410 reported cases of "systemic" or "local and systemic" reactions. The most commonly reported symptoms other than local reactions were adenopathy, fever, headache, nausea and dizziness. In 225 cases presented in table 8, the time of

TABLE 6

*Summary of Rabies Vaccine Use and Reactions in Texas from 1939 Through 1953*

| | | Brain Examined | | | | | | | | Brain Not Examined | | | | Not Given or Unknown | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Positive | | | | Negative | | | | | | | | | | | | | |
| | | Bitten | | Contact | | Bitten | | Contact | | Bitten | | Contact | | Bitten | | Contact | | | |
| | | Child | Adult | Child | Adult | Child | Adult | Child | Adult | Child | Adult | Child | Adult | Child | Adult | Child | Adult | Total | Per Cent |
| Injection Site | Hip | 73 | 21 | 48 | 20 | 3 | 2 | 9 | 0 | 48 | 15 | 4 | 4 | 4 | 2 | 0 | 0 | 253 | 3.00 |
| | Arm | 368 | 287 | 269 | 213 | 12 | 10 | 1 | 4 | 158 | 107 | 27 | 21 | 19 | 13 | 5 | 5 | 1,519 | 18.02 |
| | Abdomen | 966 | 622 | 700 | 446 | 21 | 19 | 8 | 15 | 527 | 369 | 78 | 76 | 74 | 55 | 17 | 14 | 4,007 | 47.53 |
| | Subcutaneous | 109 | 71 | 66 | 53 | 1 | 1 | 3 | 2 | 26 | 17 | 8 | 3 | 6 | 1 | 0 | 3 | 370 | 4.38 |
| | Multiple | 216 | 148 | 175 | 89 | 0 | 1 | 2 | 2 | 146 | 66 | 30 | 20 | 14 | 8 | 0 | 7 | 924 | 10.96 |
| | Not Given | 329 | 218 | 52 | 57 | 4 | 1 | 0 | 1 | 74 | 46 | 10 | 29 | 233 | 115 | 95 | 93 | 1,357 | 16.09 |
| Reactions | None | 940 | 581 | 523 | 359 | 21 | 21 | 21 | 15 | 414 | 254 | 77 | 85 | 294 | 157 | 96 | 101 | 3,959 | 46.96 |
| | Local | 1040 | 713 | 718 | 445 | 16 | 11 | 2 | 7 | 527 | 312 | 72 | 63 | 51 | 29 | 18 | 15 | 4,039 | 47.91 |
| | Local and Systemic | 36 | 37 | 24 | 38 | 2 | 2 | 0 | 2 | 12 | 19 | 3 | 3 | 0 | 5 | 0 | 5 | 188 | 2.23 |
| | Systemic | 39 | 35 | 42 | 32 | 1 | 0 | 0 | 0 | 24 | 32 | 5 | 2 | 4 | 3 | 2 | 1 | 222 | 2.63 |
| | Post-Vaccinal | 6 | 1 | 3 | 4 | 1 | 0 | 0 | 0 | 2 | 3 | 0 | 0 | 1 | 0 | 1 | 0 | 22 | 0.26 |
| | Total | 2061 | 1367 | 1310 | 878 | 41 | 34 | 23 | 24 | 979 | 620 | 157 | 153 | 350 | 194 | 117 | 122 | 8,430 | |

TABLE 7

*Analysis of Local Reactions to Injection of Rabies Vaccine*

| Reaction listed as | Number | Per cent Total 4,106 | Per cent Classified 1,152 |
|---|---|---|---|
| Local—X mark only | 2,954 | 71.94 | .... |
| Mild | 472 | 11.49 | 40.97 |
| Slight | 211 | 5.13 | 18.31 |
| Redness | 144 | 3.50 | 12.50 |
| Red after one injection | 43 | 1.04 | 3.73 |
| Red after 4 or more injections | 139 | 3.38 | 12.06 |
| Moderate | 76 | 1.85 | 6.59 |
| Itching | 5 | .12 | .43 |
| Urticaria | 3 | .07 | .26 |
| Pruritis | 2 | .05 | .17 |
| Severe | 31 | .75 | 2.69 |
| Severe or more | 26 | .64 | 2.25 |
| Totals | 4,106 | | |

The descriptions of the local reactions varied as follows:
Redness and itching, induration moderate, mild itching, redness and soreness, discoloration, two-inch red area, pain, hyperemia, few lumps remaining after injection, allergic type dermatitis at injection sites, severe last five doses, severe—needed hot compress treatment, developed a rash, etc.

TABLE 8

*Reactions Classified as Local and Systemic with no Time of Occurrence Specified*

| Symptoms | | |
|---|---|---|
| Local | Systemic | Number Occurring |
| red or red and swollen | allergic rash, local or general, slight to severe | 14 |
| redness, pain, edema | temperature, chills, malaise, headache, vertigo, joint pains | 52 |
| redness, itching, pain, edema | nausea, chills, diarrhea | 16 |
| red, swollen, pain, soreness | nausea, chills, diarrhea | 15 |
| red, swollen | malaise | 15 |
| redness | glandular involvement | 4 |
| redness | fatigue, nervousness, dizziness, stiff neck, muscle aches, blurred vision, tingling sensation in hands, amenorhea | 11 |
| redness | hospitalized—no further information | 1 |
| redness | systemic—mild moderate or severe | 37 |
| redness | asthma | 1 |
| redness | systemic—check mark only | 59 |
| | | 225 |

TABLE 9

*Recorded Systemic Reactions After One Dose and After More Than One Dose of Rabies Vaccine*

| Symptoms | After one dose | | | | | | | | After more than one dose | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1st | 2-3 | 4-5 | 6-7 | 8-9 | 10-11 | 12-14 | 14+ | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 11 | 14 | All | Total |
| Headache with fever, nervousness, vertigo, pain in knees, numbness of hands and arms, or backache | .. | .. | 7 | 2 | 3 | 4 | 1 | .. | .. | 1 | .. | .. | .. | .. | .. | .. | .. | .. | .. | 1 | 19 |
| Temperature with drowsiness, emesis, swollen arms, limped or chills | .. | .. | 3 | .. | 4 | 4 | 1 | 1 | .. | 3 | .. | 1 | 1 | 5 | 1 | .. | .. | .. | 2 | 2 | 28 |
| Nausea with headache, emesis, pain, vertigo, cyanosis and rapid pulse, abdominal cramps, emesis and diarrhea | 2 | 3 | 6 | 4 | 5 | 3 | 4 | 1 | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | 28 |
| Dizziness with drowsiness, weakness, nervousness, diaphoresis, faintness, numbness of arms, tingling sensation, insomnia, aching joints, cyanosis, soreness of neck, pain in extremities, smothering, palpitation, blurring vision, staggering gait, flushed face, diaphoresis, vertigo, malaise | 3 | 6 | 7 | 6 | 2 | 12 | 4 | .. | .. | 6 | .. | 1 | .. | .. | .. | .. | .. | .. | .. | .. | 48 |
| Glandular involvement with headache | .. | 1 | 18 | 9 | 6 | .. | .. | .. | .. | 1 | .. | .. | .. | .. | .. | 1 | 1 | 2 | 2 | 1 | 42 |
| Edema of face and rash | .. | 1 | .. | 1 | .. | 2 | .. | .. | .. | .. | 1 | .. | .. | .. | .. | .. | .. | .. | .. | .. | 5 |
| Systemic—X mark only | .. | .. | .. | .. | .. | 1 | 1 | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | 2 |
| | | | | | | | | | | | | | | | | | | | | | 172 |

reaction was not specified. In 172 cases (Table 9), 137 had reactions after a particular dose, or series of doses. Only five persons suffered a systemic reaction from the first dose, and only 38 had systemic reactions prior to the sixth dose in this particular group of cases where data was complete as to the time of occurrence of reactions. There is no evidence that any of these persons previously had received antirabic vaccine. In many of these cases the treatment was wisely discontinued when systemic reactions appeared. However, in some cases the physician unrelentingly continued the injections, and in some 35 cases systemic reactions occurred repeatedly, tending to become increasingly severe, and in several instances required hospitalization.

An analysis of the data with regard to the influence of the type of injection on incidence of reactions, as presented in table 10, showed that 5.99 per cent of those injected by the subcutaneous route developed systemic reactions, while 4.76 per cent of those

TABLE 10

*Analysis of Reactions to Rabies Vaccine with Regard to Type of Injection*

| | Intramuscular | | Subcutaneous | |
|---|---|---|---|---|
| | Number | Per cent | Number | Per cent |
| Total number receiving treatment | 336 | .... | 4,167 | .... |
| Total number showing no reaction | 157 | 46.72 | 1,446 | 34.70 |
| Total number showing reaction | 179 | 53.27 | 2,721 | 65.29 |
| Total number showing local reaction only | 163 | 48.51 | 2,471 | 59.29 |
| Number showing systemic, local and systemic or post vaccinal reaction | 16 | 4.76 | 250 | 5.99 |

P = less than 0.001.

injected intramuscularly showed such reactions. These findings were of doubtful significance. However, total reactions of all types were 65.29 per cent for the subcutaneous route and 53.27 per cent for the intramuscular route ($P = 0.001$) which suggests that the use of the intramuscular route of injection was associated with a lower incidence of reactions.

The data presented in table 11 indicated that those persons receiving the vaccine in the scapular area of the back gave the lowest incidence of total reactions, with injections over the hip, the arm and the abdomen showing slightly increasing incidence of reactions, in the order listed. However, differences were relatively slight and the influence of the site of the injection of the

TABLE 11

*Analysis of Reactions from a Selected Group of Reports with Complete Data on Site of Injection*

| Site | No Reaction | | Local | | Local and Systemic | | Systemic | | Post-Vaccinal | | Injected | Total Reactions | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Number | Per cent | Number | Per cent | Number | Per cent | Number | Per cent | Number | Per cent | Number | Number | Per cent |
| Abdomen .... | 1,046 | 35.1 | 1,766 | 59.3 | 54 | 1.8 | 110 | 3.7 | 4 | 0.1 | 2,980 | 1,934 | 64.9 |
| Arm ..... | 516 | 43.3 | 628 | 52.7 | 24 | 2.0 | 24 | 2.0 | .. | .. | 1,192 | 676 | 56.7 |
| Hip ..... | 100 | 52.9 | 85 | 45.0 | 1 | 0.5 | 3 | 1.6 | .. | .. | 189 | 89 | 47.1 |
| Back ..... | 33 | 66.0 | 16 | 32.0 | .. | .. | 1 | 2.0 | .. | .. | 50 | 17 | 34.0 |
| Multiple ..... | 256 | 34.4 | 449 | 60.3 | 17 | 2.3 | 20 | 2.7 | 2 | 0.3 | 744 | 488 | 65.6 |

vaccine on the incidence of severity of reactions was questionable.

The influence of the age of the patient on the incidence of systemic reactions did not appear to be of great significance. From the data presented in table 12, it is seen that the incidence of systemic reactions in children under 18 years of age was 4.1 per cent and in persons over 18 years of age it was 6.7 per cent. Statistical analysis indicated that this slight difference was probably not significant.

In 16 cases, or 0.19 per cent, of the 8,430 treated persons the data was definitely indicative of treatment paralysis (Table 13).

TABLE 12

*Analysis of Reactions to Rabies Vaccine with Regard to the Age of the Person Receiving Treatment*

| | CHILDREN | | ADULTS | |
|---|---|---|---|---|
| | Number | Per cent | Number | Per cent |
| Total number receiving treatment..... | 4,108 | ... | 2,773 | ... |
| Number showing no reaction..... | 2,076 | 50.5 | 1,342 | 48.4 |
| Total showing reaction ..... | 2,032 | 49.5 | 1,431 | 51.6 |
| Number showing local reaction only.... | 1,868 | 45.4 | 1,245 | 44.9 |
| Number showing systemic, local and systemic or post vaccinal reactions.. | 164 | 4.1 | 186 | 6.7 |

P = less than 0.1 and more than 0.05.

One of these cases terminated fatally. In two of these cases the saliva was tested for rabies virus, but with negative findings. All fifteen nonfatal cases eventually completely recovered. There was nothing to indicate that any of the cases reported with postvaccinal reactions had previously received antirabic treatment.

*Discussion.* Persons exposed to rabies ordinarily know when the exposure occurred and where the virus was deposited, so that the local treatment of wounds inflicted by rabid animals has long been practiced as one of the most important means for prevention or rabies. Shaughnessy and Zichis (1943) have shown experimentally that 30 per cent soft soap solution is as effective as nitric acid in this early treatment for prevention of rabies. Cautery of animal bite wounds is apparently no longer widely used (van Rooyen and Rhodes). In this present study it was not possible to evaluate the role of the local wound treatment in the prevention of the disease. According to the completed records, about 66 per cent of those who received antirabic vaccine were actually bitten by laboratory proved positive rabid animals. The location and

TABLE 13

*Cases Classified as Post Vaccinal Reactions to Rabies Vaccine for Five-Year Period 1949 Through 1953 (One Fatality)*

| Patient Age | Sex | Number Doses* | Type of Exposure Animal | Exposure | Reactions‡ |
|---|---|---|---|---|---|
| 31 | M | 10 | rabid cow | saliva on superficial hand cuts | 11 days after 1st dose fever; aching; severe nausea; marked weakness; prostration; urinary retention; death. Autopsy report—encephalitis. |
| 30 | M | 14 | rabid dog | laceration on hands | Probable encephalitis—nuchal rigidity; deep reflexes absent at rt. knee and ankle; temperature 101° F. |
| 50 | F | 14† | rabid dog | contact | Reaction to 4th and 5th doses; 19 days after 1st dose weakness and collapse of legs; severe pain in back, arms and legs; urinary retention; nausea; emesis; hysteria. |
| ? | M | 10 | lost dog | bites on legs | Nausea and fatigue after 3rd dose; 13 days after 1st dose urticaria on extremities; nausea; numbness of legs; urinary retention; clonic contractions of legs. |
| 31 | F | 9 | rabid dog | bite on thumb | After 10th dose nausea and dizziness; severe pain at base of head; temperature 102° F. treatment stopped; hospitalized; urinary retention; loss sensation to touch; extreme weakness; slight paralysis of legs. |
| 9 | M | 13 | rabid dog | exposure on legs | After 13th dose developed acute symptoms of toxic myelitis; hospitalized 10 days. |
| 20 | M | 14 | rabid human | contact sister | After 8th dose enlarged axillary glands; after 11th dose cyanosis; diaphoresis; twitching of arms and head. |
| 7 | F | 11 | rabid dog | fed dog with cuts on hands | Large local reaction after 8th dose; 14 days after 1st dose headache; legache; emesis; fever; treatment stopped; difficulty in walking continued several weeks. |
| 52 | M | 12 | rabid dog | handled | 12 days after 1st dose severe rash over body; fever; headache; severe anaphylaxis. |
| 32 | F | 14 | rabid dog | contact | After 11th dose faintness; seizure-type reaction; incontinence; rigidity; stertorous breathing; no history of previous seizures. |
| Adult | F | 7 | rabid dog | contact | After 4 days severe rash on body; treatment stopped; hospitalized; ACTH cleared up rash. |
| 59 | M | 9 | stray dog | bite on leg | Physician checked as post-vaccinal reaction; hospitalized on 18th day; no other information. |
| 11 | M | 18 | stray dog | bite on face | Sleepy at 19th dose; pain in head and legs; weakness left side and arm; hospitalized; treated with ACTH; completely recovered. |
| Adult | F | 13 | rabid dog | contact | At 13th dose temperature 104° F.; hospitalized; encephalitis; treated Acthar Gel; released in 9 days progressing satisfactorily. |

TABLE 13—Continued

*Cases Classified as Post Vaccinal Reactions to Rabies Vaccine for Five-Year Period 1949 Through 1953 (One Fatality)*

| Patient Age | Sex | Number Doses* | Type of Exposure Animal | Exposure | Reactions‡ |
|---|---|---|---|---|---|
| 6 | F | 14 | rabid skunk | bite on hand | Severe post-vaccinal reaction; encephalomyelitis with residual paralysis back, adbdomen; and all extremities; improving but still at hospital 6 months later. |
| 1½ | M | 14 | stray dog | bite on hands | Mother stated "child fell when it walked" after 1st and 5th doses. |
| 4 cases and 1 case | | 14 14 | rabid dog negative dog head | contact bite | Physician checked as post-vaccinal. No other information available. |

* Semple phenol-treated rabies vaccine of Texas State Department of Health.
† Commercial rabies vaccine.
‡ One fatal reaction; all others recovered; one with sequelae.

the severity of the wounds in this series of treated persons varied greatly, with a considerable number being reported multiple and severe. The mortality from rabies is thought to depend particularly on the quantity of the virus inoculated, the site of the injury, the degree of trauma, and the virulence of the virus. It was interesting to note that in twelve deaths from rabies in only one instance was the bite wound classified as severe. This one case was in military personnel and the wound was a deep bite wound on the face and it was given immediate treatment in the first-aid center. Sellers (1953) in an analysis of the ratio of deaths to persons treated and the location of the wound reported a ratio of 1:160 for face wounds, 1:1800 for hands, 1:4350 for body wounds, and 1;6670 for wounds on the legs and feet. Since it is expected in a large series of rabies treatments to encounter vaccine failures and since none appeared in 8,430 persons included in this study, it seemed that the vaccine treatment was an effective preventative measure. Even when we consider the twelve known deaths from rabies, all in unvaccinated persons, we feel that the mortality rate in persons who suffered bites by rabid animals is much less than the 5 to 15 per cent mortality rates usually cited (Cornwall, 1923, and Yu, 1941).

As Sellers (1948) has so well stated, it is not hydrophobia but "rabiphobia" which constitutes the major and most troublesome problem to the practicing physician and health officer. The ad-

ministration of antirabic vaccine to persons actually bitten by known or strongly suspected rabid animals is a justifiable procedure in that danger of the disease is greater and more serious than any ill-effect of the vaccine. However, physicians are prone to overlook this fact that the danger of treatment far exceeds that of rabies and "rabiphobia" in presumed exposures other than those actually occurring through bites by known or strongly suspected rabid animals. Sellers (1948) has reported that of 50,000 persons taking antirabic vaccine, treatment paralysis occurred at the rate of one in 7200. The treatment paralysis rate cited by McKendrick (1940) for a larger series was one in 3,500 persons treated with desiccated cord vaccine and one in 8,500 persons treated with phenolized vaccines. Applebaum, Greenberg, and Nelson (1953) reported a treatment paralysis rate of one in 2,025 persons treated. In Los Angeles County, California, during 1940–45, a rate of treatment reaction of one in 1,194 persons was cited (Redewell and Underwood, 1947). However, in the present series of 8,430 persons treated, the severe post-vaccinal reaction rate was one in 527 or one in 870 in the 13,925 treatments distributed and used (Table 1). Various theories of the cause of treatment paralysis have been advanced, with the allergic response theory seeming to be the most plausible. However, the possibility of modified rabies infection should not be entirely overlooked. The lighting-up of poliomyelitis or other latent viral infections is also a possibility to be considered in studying these cases of reaction to rabies vaccine.

The avianized rabies vaccine (Tierkel, *et al.*, 1949) which is now being widely used in immunizing animals would appear promising in overcoming some of the objections to the present human vaccines. The use of hyperimmune serum also offers hope for a more effective prophylaxis of rabies virus infection.

*Summary.* During the period 1949 through 1953, twelve human deaths from rabies, one of which was attributed to the bite of a rabid bat, occurred in Texas. None of these patients had received antirabic treatment. Since there were no deaths from rabies in a group of 8,430 persons receiving antirabic vaccine following exposure to rabies, and it is expected in such a large series to encounter treatment failures, it appeared that the vac-

cine treatment was an effective measure in a state where rabies was shown to be endemic.

Treatment paralysis or post-vaccinal reaction occurred at the rate of one in 527 treated persons in the group of 8,430 treated with phenolized rabies vaccine. The use of the vaccine is not at all entirely a harmless procedure and should be restricted to persons definitely at risk of rabies infection.

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# EFFLUENT CONSTRICTIONS IN THE RENAL VASCULAR SYSTEM*

H. L. Koester, J. C. Locke, and H. G. Swann

The pressure within the renal vein and some of its tributaries has been ascertained (Swann, Hink, Koester, Moore, and Prine, 1952). In the arcuate veins or in the larger cortical veins, it is found to be about 25 mm. Hg; but in the interlobar veins, only a millimeter or so below the arcuate site, the pressure suddenly drops to about 7 mm. Hg. The experiment suggests that some resistance or constriction—a dam as it were—must lie between the two points, *i.e.*, close to the arcuate-interlobar junction. Furthermore, if there were such a dam, blood must pile up behind it. This in fact is known to be true, for the kidney is normally distended with fluid: when its artery is suddenly occluded, there runs out of the kidney a volume of fluid equal to about 22 per cent of its normal functioning volume (Swann, 1952; Swann, Feist, and Lowe, 1955). With these facts in mind, we have examined closely the finer morphology of the veins of the kidney to ascertain whether any structures resembling effluent constrictions may be found in them.

Thorough descriptions of the renal venous system were given by Hauch (1904), v. Mollendorf (1930) and Leins (1935). The venous blood from the deeper portions of the renal cortex drains either (1) outward into the superficial cortical veins beneath the renal capsule or (2) directly toward the hilus by way of the cortical profunda veins (Golubew, 1893; Hauch, 1904). The superficial cortical veins converge beneath the capsule to form the stellate veins (Clara, 1938–1939). Here, extrarenal anastomoses between the stellate and capsular veins are present (v. Mollendorf, 1930; Spanner, 1937), and in some instances, this drainage may in compensation become large enough to carry the entire venous drainage of the kidney (Langley, 1925; Johannson,

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1926). Occasionally, large bundles of smooth muscle are found at the point of convergence of the stellate veins of man (v. Mollendorf, 1930), which could possibly influence the route of drainage at this point. The "interlobular" veins, including the cortical profunda veins and branches from the stellate veins, pass radially through the cortex to empty into the arcuate veins at the corticomedullary junction. These interlobular veins are extremely thin-walled, being composed essentially of a single layer of elongated endothelial cells which are supported by a sparse network of elastic and reticular fibers (v. Mollendorf, 1930; Sestini, 1938). In human kidneys, occasional isolated smooth muscle fibers may be found in these vessels where the supporting elements are more abundant (v. Mollendorf, 1930).

The medulla of the kidney is drained by the venae rectae, which arise from the capillary network in the renal papillae and take a straight course toward the corticomedullary junction to drain into the convex (cortical) surface of the arcuate veins (Leins, 1935).

The drainage of both the cortex and medulla, therefore, is primarily into the arcuate veins. (We have noted, however, that in many instances—and this is particularly true of the dog kidney—interlobular veins may drain into upper interlobars, without passage through arcuates.) As a rule, the arcuate veins lie on the medullary side of the arcuate arteries. The walls of these veins are thin on the cortical side, but they have a large amount of supporting tissue, containing longitudinal smooth muscle, on their medullary side; this gives the appearance of an eccentric lumen on cross-section (v. Mollendorf, 1930). The arcuates have extensive anastomoses with one another at the base of the pyramids (Morrison, 1926). These vessels converge and leave the renal parenchyma by forming the interlobar veins; the latter lie in the renal sinus.

The interlobar veins are the first venous channels encountered which have a definite tunic of longitudinal smooth muscle. They are embedded within the loose adipose connective tissue of the renal sinus and converge to form the larger tributaries to the renal vein. The main renal vein has a pronounced muscular tunic, composed largely of longitudinal smooth muscle (Hochrein and Singer, 1927).

Franklin (1927) discusses the valves of the major renal veins; some find them present, but a few deny their occurrence. Valves are said to be absent in the veins above the renal hilus (Golubew, 1893; Leins, 1935). In the human fetus, Kampmeier and Birch (1927) describe valves within the renal veins; in addition, the fetus has a conspicuous, sphincter-like structure just at the confluence of the renal veins with the vena cava. In avian kidneys, Spanner (1925) described valve-like structures in the renal-portal venous system. These were demonstrated by Sperber (1948) to be functional, with contraction evidently mediated by cholinergic fibres (Rennick, 1954).

Smooth muscle in the veins of the kidney above the renal sinus is described only very infrequently. Durante (1928), v. Mollendorf (1930) and Sestini (1951) all refer to it briefly, and their reports will be discussed below. Spanner (1937) claims that sphincters are found in conjunction with arterio-venous anastomoses; he reports that in human kidneys, there are anastomoses between interlobar vessels, between interlobular vessels and between capsular vessels. Arcuate anastomoses were first described by Golubew (1893); associated with them, according to Barrie, Klebanov and Cates (1950), is a plexus of sinuses which they designate as "arcuate sponges." These also will be discussed in greater detail below.

*Methods.* In the study of the renal venous morphology, two species were utilized: man and the dog. The dog kidneys were obtained by careful dissection and removal of the kidney while the dog was under sodium pentobarbital anesthesia. The animals were all in good health and ranged from five to fifteen kilograms in weight. All human kidneys were obtained at necropsy from adults who had no demonstrable renal disease. In studying the venous system, three approaches were utilized. The first, that of semi-microdissection, using a binocular microscope at magnifications of X2 to X10, was found to give a lucid picture of the morphological configuration of the inner surfaces of the venous vessels in man. The kidneys were prepared by slicing them longitudinally into halves and fixed in 4 per cent neutral formalin. Dissection was begun using a variety of small instruments, the field being well illuminated and magnified. The hilar vessels were traced back into the kidney parenchyma by incising longi-

tudinally along the walls of the veins. The parenchyma was sliced away from the interlobar and arcuate veins so that when slit, their walls could be laid back and the ostia of their tributaries observed. The preparation was periodically moistened to prevent drying. Because of the extreme fragility of the intrarenal veins of the dog, semi-microdissection proved impractical in this species.

In studying the histology of the intrarenal veins, small portions of renal tissue, including the arcuate-interlobar junction, were excised, fixed in formalin and embedded in paraffin. Sections were cut at thicknesses varying from 6 to 15 micra. Most sections of the dog kidneys were cut at 6 micra; whereas, those of the human kidneys were cut at 15 micra. For staining the sections, the trichrome method of Milligan (1946), consisting of acid fuchsin, orange G and light green, was used. This staining technic was chosen because it gives a vivid differentiation between smooth muscle and connective tissue and at the same time is simple, rapid and dependable. For survey, each tenth section was mounted and stained, and serial sections were made in particular areas under investigation.

Latex rubber casts were also made of the venous system of dog kidneys, following the method of Lieb (1940) and Duff and More (1944).

## *Results*

In both human and dog kidneys, two structures were found in the veins which might act as effluent constrictions. They will be described with the terms "stenoses" and "sinusoidal cushions"; good examples of them are shown in figure I. Because they are slightly different in the two species investigated, the findings for each will be reported separately.

I. *Stenoses: human.* In human kidneys, the stenoses were originally observed during semi-microdissection of the veins, and indeed this method still furnishes us with one of the best means of observing them. As the veins are traced back, slitting their walls longitudinally, the stenoses are first seen at the ostia of smaller tributaries entering larger veins. In structure, they are, in essentials, a narrowing of the ostial opening just at the point where the smaller vein debouches into the larger. One can readily run a

fine hook through the ostium, using strong illumination and some magnification, and pull back the lips of the ostium to examine the wider tributary beyond the stenosis. In those shown in figure I, the narrowing is caused by a single lip. In others, the stenoses are like iris diaphragms, with their ostia now well-centered and now quite eccentric. Figure II shows sketches of the variations in structure which we have observed.

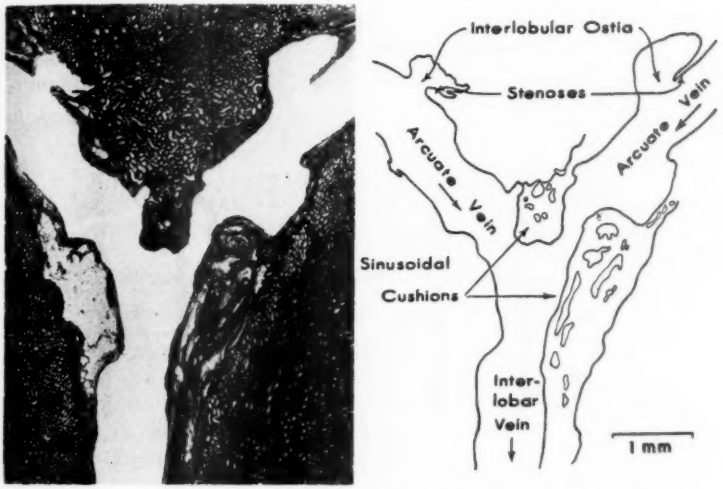


FIG. I. Stenoses and sinusoidal cushions in the renal veins. Two stenoses are visible at interlobular venous ostia. The sinusoidal cushions lie at the confluence of two arcuate veins with an interlobar vein. Human.

The position of the stenoses is as follows: they are common at the ostia of *smaller* tributaries entering the interlobar veins—such tributaries might properly be called interlobular veins rather than arcuates (see Barrie, Klebanov, and Cates, 1950). Where a group of arcuates and other large tributaries enter the interlobars, stenoses are not clearly apparent, because so many vessels are apt to converge that one cannot say that the venous channel truly narrows at any one point. But narrowing is apparent in histological sections of these sites, as will be discussed below. As one dissects the arcuates above a major confluence, the

stenoses again become evident at simple interlobular-arcuate junctions.

It is to be stressed that these stenoses are not true vein valves: they are not shaped in cusps, and their structure is such that they cannot block retrograde blood flow. Physiologically also, they cannot block flow, for one can easily inject blood or latex rubber backwards through them. We (Koester, Swann, and Locke, 1953) previously called them "check-valves"; the name is unsuitable, because a valve usually blocks flow of fluid. The more apt and precise term is that of stenosis.

On histological examination, the stenoses are seen to be composed of a dense collagenous framework, lined with endothelium.

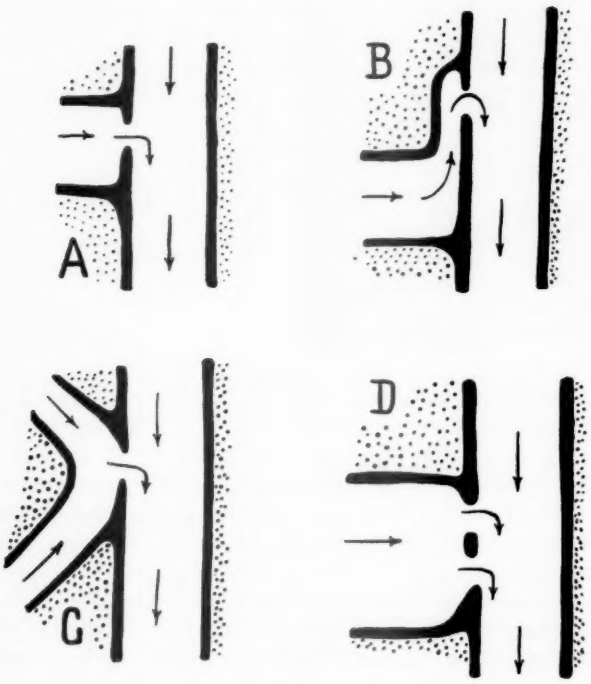


FIG. 11. Various types of stenoses. The narrowing at the ostium is thought to form an effluent constriction. Sketch D shows a bar across the ostium. In human kidneys, these stenoses are often irregularly laced with smooth muscle.

Sometimes they contain no smooth muscle (see the two stenoses in figure I), but much more frequently, muscle is present in quantity (Fig. III). It appears to be circular and is apparently a

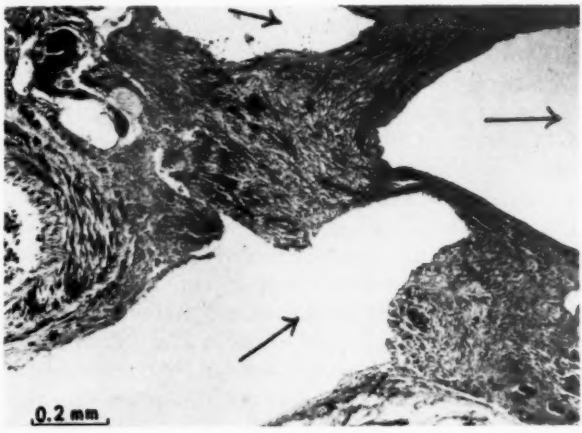


FIG. III. Smooth muscle in venous stenoses. The muscle appears black (see arterial media on left) and connective tissue appears gray. Two tributaries are just about to enter an interlobar vein at the right. Human.

proliferation of the media, which is absent or very poorly developed in this region except at these sites. At the interlobular-arcuate junctions, the muscular components are sparse. But at the ostia of tributaries entering the interlobar veins, the stenoses are richly supplied with muscle so that they appear like purse-string sphincters. Where several large veins converge into a larger channel, the supporting tissue is more abundant and the adventitial smooth muscle bundles coalesce so that an interlacing network of smooth muscle fibers appears around the point of confluence (Fig. III). This lacework of smooth muscle was apparently observed in part by Sestini (1951). He briefly reported that it lies around the arcuate veins; actually, its distribution is more extensive than this. In our series, it is particularly prominent at the arcuate-interlobar junctions. Durante (1928) reported observing "sphincters" in the veins of the kidneys. Examination of his photomicrographs (1929) suggests that these "sphincters" are the same as the longitudinal smooth muscle

bundles which v. Mollendorf (1930) subsequently described as proliferations occurring along the medullary side of the arcuate veins. The structures observed by these last two workers are evidently different from the interlacing network under discussion.

Another structure occasionally seen during semi-microdissection is a bar of tissue traversing the lumen of the vein (Figs. II and IV). Such bars appear in human but not in dog kidneys. They have been seen at various sites in the human kidney, from major tributaries of the renal vein up to veins in the corticomedullary region. They are often found crossing the ostium of a larger vein just beyond the point of drainage of a smaller tributary. Careful examination of all the veins in a sample of tissue gave the estimate that there are roughly a dozen such bars in a single kidney. On microscopic examination, these bars are found to be endothelial-covered structures, containing a framework of elastic and collagenous tissues. Within the connective tissue, heavy bundles of smooth muscle are seen to run parallel to the central axis of the bar and to flare out at each base to join with the muscular tunic of the vein wall. If contracted, they would pull the vein into an elliptical shape. Structures similar to these have been described within the orifices to the marginal sinus of the human placenta (Spanner, 1935) and within the lumen of vessels forming arterio-venous anastomoses in the cock's comb (Staubesand, 1951). The mode of origin of these unusual structures is postulated to be as follows: in the formation of veins from capillary networks, incomplete fusion of the primitive vascular channels may leave remnants crossing the lumen. With further development of the veins, these remnants assume the appearance previously described for the bars.

*Stenoses: dog.* In the dog's kidney, stenoses of fundamentally the same form and structure are encountered. They are revealed best in latex rubber casts of the kidney veins, appearing as sharp indentations partially encircling the cast. Occasionally the encirclement is complete thus showing the negative image of a stenosis which is shaped like an iris diaphragm. Figure V shows a latex cast of veins having these stenoses. The sharp indentations in the molds of the venous channels appear like wrinkles in the bent fingers of a partially inflated rubber glove. Significantly, they are found in the latex casts regardless of the injection pres-

FIG. IV. Bar across primary tributary of renal vein. The vein wall is laid open and a hair put on the distal side of the bar. The invisible ostium of a large tributary lies behind the bar.

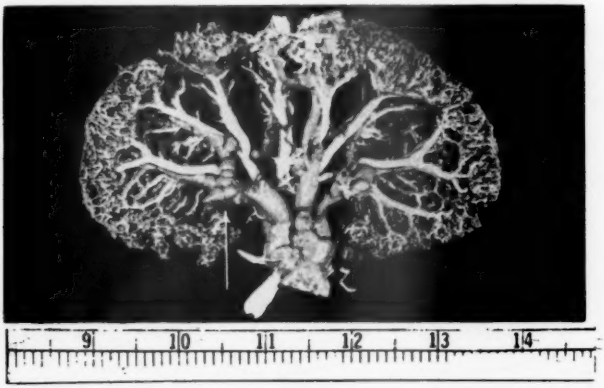


FIG. V. Latex cast of arterial (white) and venous (gray) trees of a dog kidney. Just above the point of the arrow there are several stenoses in an interlobar vein. This is unusual: the stenoses are customarily located higher up in the venous system.

sure: they are seen as readily if 20 mm. Hg is used to inject the latex as if 100 or 200 mm. Hg is used. This shows that they are not the wrinkles which might appear when a vein collapses. Their variation in structure is like that in human kidneys, as diagrammed in figure II. In the dog, stenoses appear occasionally along the course of the interlobars and primary tributaries to the renal vein; however, they are present primarily at the confluence of arcuates with interlobars and of interlobulars with arcuates.

Histological examination shows the stenoses in dog kidneys to be composed primarily of elastic and collagenous tissue. They are broadly affixed to the vein wall at their base, then taper progressively toward the ostium, sometimes becoming membranous and consisting of only two endothelial layers supported by a thin collagenous and elastic network (Fig. VI). Smooth muscle is very inconspicuous in the stenoses of the dog kidney, in sharp contrast with its distinctness in human kidneys.

II. *Sinusoidal Cushions: human.* A second type of apparent constriction in the venous effluents has been observed by microscopic study of serial sections. It can be readily seen that the connective tissue continuation of the kidney capsule, which lines the renal sinus, extends peripherally to form the supportive tissue of

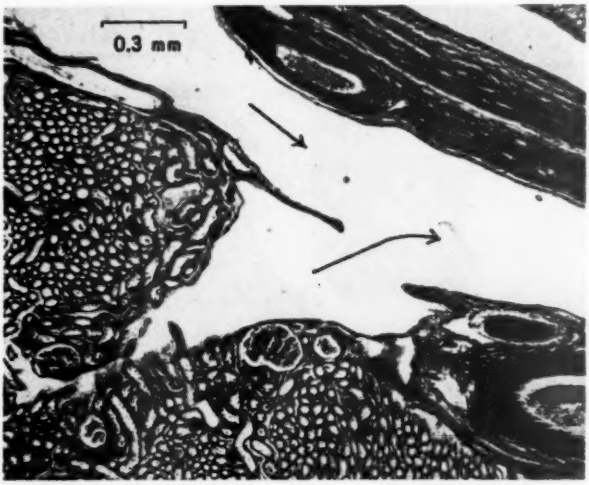


FIG. VI. Stenosis at the ostium of an interlobular vein. Dog.

the vessels within the parenchyma. At the base of the pyramids, there are augmentations or cushions of this tissue and within them lie numerous, thin-walled venous sinuses. They are illustrated in figures I, VII and VIII; the "spongy" appearance of the sinus-filled cushion, which led Barrie, Klebanoff and Cates (1950) to call them "arcuate sponges," is particularly apparent in figure VII. Like the stenoses, these structures are often laced with smooth muscle, both close to their surfaces in a sphincter-like system and also deep within the connective tissue matrix. In figure VII, the numerous deeply staining aggregates of muscle are evident; in figure VIII, only a few are to be seen.

At the confluence of arcuates with one another to form an interlobar vein, the sinusoidal cushions usually appear. Such cushions may actually surround the perimeter of the vessel. Sometimes they are seen, as illustrated in Figure VIII, at the confluence of interlobulars with arcuates. In many sections, the lumen of the veins at the arcuate-interlobar junction appears narrowed because of the encroachment upon their outer walls by the apparently engorged sinusoidal cushion (Fig. VII). The morphological configuration of the kidney is such that the venous drainage courses within the narrow confines between the bases of the pyramids; hence the imposition of these large sinusoidal

FIG. VII. Sinusoidal cushions in human kidney. This is a high magnification of figure I. Numerous sinusoids are visible in both cushions.

cushions might further encroach upon this already restricted area. It is difficult to trace the source or drainage of these large venous sinuses. They are seen to connect with both interlobular and medullary veins by small, very muscular venous channels. On several occasions, an apparent connection between small tortuous arterioles and the venous sinuses has been seen. The whole structure is strongly suggestive, as will be discussed below, of the so-called "erectile tissue" of the nasal mucosa.

These venous sinuses lying in the connective tissue were observed by Spanner (1937) and by Barrie, Klebanoff, and Cates (1950). Spanner (1937) describes them as isolated accumulations of large venous plexi arranged superficially along the walls

of the minor calices. He finds them to be pelvic veins and considers that they form arterio-venous anastomoses between the interlobar vessels. The "arcuate sponges" described by Barrie, Klebanoff, and Cates (1950) are undoubtedly the same sinusoids. Since these workers did not distinguish between arcuate and interlobar vessels, the exact location of their "sponges" is uncertain. In both reports, however, these sinusoidal spaces are said to be associated with arterio-venous anastomoses and consequently would be at times engorged with blood under relatively high pressures.

*Sinusoidal Cushions: dog.* In the dog, the sinusoidal cushions are also present (Fig. IX); however, they are far less extensive

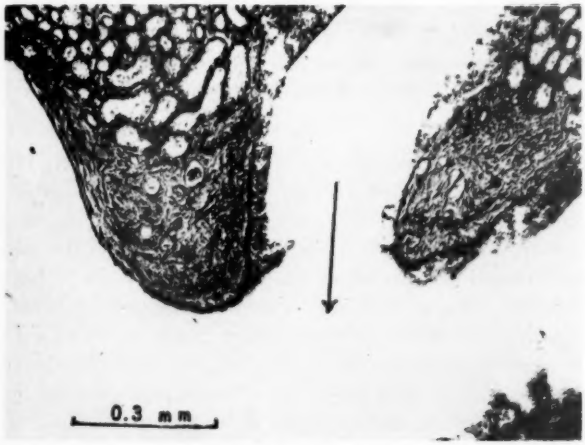


FIG. VIII. Sinusoidal cushions in human kidney at junction of interlobular and arcuate veins.

than in the human kidneys. Here again, they lie primarily close to the arcuate-interlobar junction. In the material of this study, they were frequently collapsed and difficult to visualize. As with the stenoses in the dog, smooth muscle in the cushions is very inconspicuous or absent.

*Discussion.* As described in the introduction, the fact that the venous pressure in the arcuate vein is about 25 mm. Hg., whereas that in the interlobars is only about 7 mm. Hg., led to the hy-

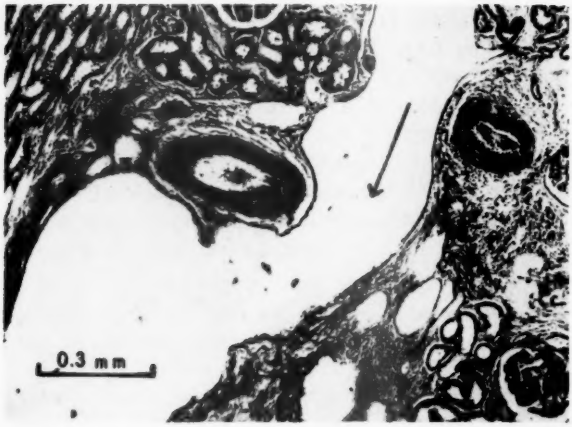


FIG. IX. Sinusoidal cushions near junction of arcuate vein with interlobar vein in a dog kidney. Sinusoids are visible above the central artery and below the arrow point.

pothesis that some resistance must lie between the two regions in the renal vasculature. In this paper, we have described two structures which might serve as these resistances, the stenoses and the sinusoidal cushions. In the human kidney, the stenoses contain smooth muscle, which suggests that they are perhaps adjustable and may be tightened down or loosened up, thus controlling the rate of blood flow through them. Increasing the effluent constriction would also, of course, lead to further engorgement of the kidney with blood and a consequent swelling in size. Indeed, it has often seemed likely to us that a vasodilatation of the kidney might result not only from arterial dilation but as well from venous constriction. In any event, the question of vasodilator fibers to the kidney is still a vexed one (Smith, 1939); in some conditions, apparently, they have been demonstrated, in particular by Rose-Bradford (1889). In the case of the paradoxical and unusual vasodilating effect of epinephrine, originally thought to involve the efferent arteriole (Richards and Plant, 1922), Smith (1951) suggests that the hormone may act at some point in the renal venular system well beyond the efferent arteriole. Such a point might very well be at the stenoses in the venous effluents.

The resistance known to be near the arcuate-interlobar junction might also be furnished by the sinusoidal cushions: they might swell and push against the veins, thus throttling down the flow of blood out of the kidney. As noted above, these sinusoidal cushions resemble the "erectile" tissue of the nasal mucosa. The latter is said to become engorged with blood by the following mechanism: arterial shunts lead directly into the sinusoidal spaces. When they are dilated, the sinuses fill with blood. Furthermore, flow of blood out of the sinuses is slowed by means of contractions of the small, heavily-muscled veins draining the sinuses. The mucosa, then, is engorged with blood by a simultaneous arterial dilatation and venous constriction (Dawes and Pritchard, 1953). The presence in the kidney also of the same group of structures has been described above: arterial shunts directly into the sinusoids, the sinusoids themselves and muscle around their draining veins. They may be presumed to act in the same way as in the nasal mucosa.

Finally, in addition to the two devices for effluent constriction discussed above, there is still a third possibility. The very thin-walled interlobular veins might have in them functional constrictions similar to those probably present in the vortex veins of the eye (Swann, 1954). Functional constrictions like these may readily be demonstrated by subjecting a length of light rubber tubing to a uniform external pressure. When water from a reservoir is run through the compressed tube, a constriction is formed at the extreme distal end of the rubber tube, just at the boundary where it leaves the imposed external pressure. The constriction is "functional" in the sense that it depends upon the hydrodynamics of the system; it is not present unless fluid is flowing through the tube. We have previously postulated that this is the primary mechanism involved in the effluent constrictions of the kidney: where the thin-walled parenchymatous veins suddenly empty into the thicker-walled extraparenchymatous (interlobar) veins, a functional constriction would presumably form (Swann, 1952).

We have discussed three structures which might form effluent constrictions: stenoses, sinusoidal cushions and functional constrictions. Of the three possibilities, we favor the hypothesis that it is the stenoses which play the major role. They are present and

conspicuous in both human and canine kidneys; whereas the sinusoidal cushions are not obvious in the dog. Moreover, the effluent vessels of the kidney do not possess a conspicuous lymph channel surrounding them, such as occurs in the eye's veins, this being a necessary morphologic adjunct to the formation of functional constrictions. Therefore, in summary, it now appears most likely to us that the structures furnishing the main effluent resistances of the kidney are the stenoses. But regardless of which structure is the critical constrictor, the physiologic and morphologic data are harmonious: the former show that some constriction must be present at a certain point and the latter reveal obvious constrictions at exactly the required point.

The morphology and sites of the stenoses account for some experimental facts about renal vein catheterization which were hitherto unexplicable. It has been noted that retrograde catheterization of the arcuate or interlobular veins, the catheter being run blindly up the main renal vein, is difficult, for we fail in about half of our attempts (Swann, Hink, Koester, Moore, and Prine, 1952). In such failures, we find the tip of the catheter still in the interlobars, and further pushing fails to advance it. It is apparent that the tip either fouls in a crooked vein or actually catches in one of the stenoses, thus preventing any higher advance of the catheter. We have also noted that when the catheter is withdrawn a millimeter at a time, the pressure at its tip sometimes falls precipitously from, say, 25 mm. Hg. to 7, but at other times falls in steps, *e.g.*, from 25 to 17, then from 17 to 7 (Swann, 1955). It is now apparent to us that in such cases the tip is passing through regions containing serial stenoses—perhaps one at an interlobular-arcuate junction and another in upper interlobar veins (Fig. V). The pressure, then, drops in serial steps as the catheter's tip passes through each constriction.

We have previously shown that the kidney is functionally distended with fluid. This fluid is two-thirds blood and one-third a fluid suggested to be tubular urine (Swann, Feist, and Lowe, 1955). In another paper will be reported some measurements of the distribution of the distending blood: about one-third of the total in the arterial tree, about one-third in the interlobular veins and about one-third in the arcuate and larger veins. This distension is functional, being dependent on the blood pressure: when

one abolishes the blood pressure, the kidney collapses. Evidently, the kidney is comparable to a balloon placed between influent and effluent tubes and inflated by a moving column of fluid. In such a system, there must be considerable resistance in the effluent tube to accomplish any inflation at all, and it is the stenoses (sinusoidal cushions?) which furnish the effluent resistance for the kidney. They are the dams in the stream, for which there is good physiological evidence. If the dams were not there, then there would be no lake, *i.e.*, the kidneys could not be functionally distended with blood.

The physiological meaning of the functional distension is still under investigation. It is our present hypothesis that it confers on the kidneys its very low resistance to blood flow. Although the organ has a double arteriolar and double capillary circulation, which might be expected to cause a great resistance to blood flow, the flow is actually very large, *e.g.*, some four times greater than that through the heart muscle, an organ of about equal size (Glasser, 1950). But with the functional distension, the smaller vessels of the vascular system would become comparatively wide in bore, and hence the factor of wall-friction, particularly great in tubes of very small bore, is much reduced. The kidney, therefore, can be considered to be in a state of constant vasodilation, with an associated very small resistance to total blood flow.

The comparative morphology of renal effluent constrictors is of considerable interest to us. As mentioned above, both the human fetus and birds have specialized constrictions in the effluent drainage of their kidneys: in the fetus, a muscular sphincter in the renal vein at its entrance into the vena cava (Kampmeier and Birch, 1927) and in birds, a valve-like structure in the renal-portal system (Spanner, 1925). In both species, these structures may be presumed to participate in a functional distension of the kidney.

Structures like the stenoses have been described in the maternal placental veins. They have been designated by various imaginative terms: Spanner (1935) says they resemble stops in a flute; Falkiner (1939) says that they are "port-hole-like." In the maternal placenta, bars of connective tissue are also thrown across some vein lumina (Spanner, 1935). Furthermore, in the fetal placenta, sphincters around the veins are common: Span-

ner (1935) calls them "Drosselvenen" or throttling veins; Danesino (1951) calls them "blocco" or blocking mechanisms. In our opinion, the presence in both the kidney and the placenta of such similar structures is more than mere coincidence: we view them as the morphological complement of physiological distension of the two tissues, the placenta being even more engored with blood (Barcroft, 1946) than is the kidney. One of us, in fact, found the "intraplacental pressure" to be about 26 mm. Hg. (Locke, 1953) just as it is in the kidney (Montgomery, Davis, Prine, and Swann, 1950). Such functional distensions are possible, in our view, only if effluent constrictions are present in a given tissue; and effluent constrictors are in fact known to be present in many tissues which are, or may be, distended with blood: penis (Deysach, 1939; Conti, 1952); nasal mucosa (Dawes and Pritchard, 1953); cock's comb (Staubesand, 1950); salivary glands (Spanner, 1937); spleen (Robinson, 1930; Herrlinger, 1949); liver (Tischendorf, 1939; Thomas and Essex, 1949) and a great many other tissues (see Märk, 1941). A review of these effects—we have called them "prethological," from $\pi\rho\eta\theta\omega$ for "I blow up" or "I swell by blowing"—will be given in another report.

*Summary*. Two structures have been identified in the renal venous system of man and the dog which are postulated to subserve the function of renal effluent constrictors: first, stenoses, located at the junction of interlobular with arcuate veins or close to the junction of arcuate with interlobar veins. These structures are abrupt narrowings of the vascular bed just at the point where tributaries drain into larger channels. In shape they resemble iris diaphragms, with the hole now well-centered and now eccentric. Histologically they consist of a dense collagenous framework lined with endothelium. In man they are often laced with a network of sphincter-like smooth muscle which, if contracted, would narrow the ostium of the tributary still further. In the dog, smooth muscle is very inconspicuous in them.

The second structure has been designated by the term "sinusoidal cushions." They usually lie close to the junction of arcuate veins with interlobar veins, but occasionally they may be found higher in renal venous system. These structures are cushions of connective tissue, pierced with numerous large thin-walled sinusoids. They resemble the "erectile tissue" of the nasal mucosa.

In man, smooth muscle is laced through and through them, both close to their surfaces and also deep within the connective tissue matrix. In the dog kidneys, sinusoidal cushions are present but less extensive than in man, and they contain virtually no smooth muscle.

These two structures are postulated to be responsible for the functional distension of the kidney: as effluent constrictors they induce the formation of a lake proximal to them in the renal vascular system. The high pressure in the interlobular veins, similarly, is thought to be dependent upon their presence.

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# THE CHARLESTON MEDICAL METEOROLOGISTS*

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Through the kindness of Eric Miller, former U. S. meteorologist stationed at the University of Wisconsin, I have recently had in my hands a thesis by Robert Croom Aldredge on "Weather Observers and Observations at Charleston, South Carolina, 1670–1871." I know nothing of Mr. Aldredge, other than that he wrote an extraordinarily interesting paper. It was printed, minus most of the illustrations, in the Historical Appendix of the Year Book of the City of Charleston for the year 1940. What I have to say here must necessarily be in large part frank copying of his words. There are no other sources than those he quotes.

The weather has long been related in the popular mind to ones state of health. Several prominent medical men have in recent years made considerable effort to put such a relationship on a sound scientific basis. I have no idea how far back in time one might find references to the subject but certainly the ancients had their health centers sometimes chosen because of favorable weather conditions.

Medical men have therefore always shown some professional interest in the weather. Many diseases have been found to reach a peak in number and virulence at certain seasons of the year. That in many of these cases the increased incidence was due primarily to favorable conditions for a vector was of course not known to early workers.

The first man in America to carry out a systematic study of the effect of meteorological conditions on the human body was a Dr. John Lining of Charleston. The basis of his work was careful weather observations which he then tried to correlate with human physiology and the cause of epidemic diseases.

It is quite understandable why this interest should develop in a Carolinian. The Proprietors of the Province of Carolina were faced with the problem of making the attractions of the country known. These were of course a fruitful land and a healthful cli-

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mate. Anything short of this would not attract settlers and settlers' meant wealth to all concerned. Quite similar to the enticing folders now gotten out by our energetic Chambers of Commerce were the Promotion Pamphlets of the 17th century which extolled the physical beauty and virtues of coastal Carolina. In one of these we find the following persuasive description:

". . . . the air comes to be considered, which is not the least considerable to the well being of a plantation, for without a wholesome air all other considerations avail nothing; and thus it is which makes this Place so desirable. . . . The summer is not too hot and the winter is very short and moderate but agreeing with English constitutions. . . . A remarkable instance of the Healthfulness of the Place is, that at the first setting down of the colony, when they had no house nor harbour, but wrought hard all day, in preparing wood to build, and lay in the open air all night, yet not one of them was ill, but continued well all the time.

If therefore any industrious and ingenious persons shall be willing to partake of the Felicities of this country, let them embrace the first opportunity, that they may obtain the greater advantages."

But Carolina was not quite paradise and the pamphleteers soon found themselves busy combating the rumors of fevers, agues, insects, pestilential marshes, hot summers and cold winters. The explanations volunteered proved the truth of some of the conditions mentioned although an attempt was made to show they were unimportant and rare. Mr. Wilson, Secretary to the Lords Proprietors, in 1682 admitted that seldom did a winter fail to produce ice on the Ashley River. It was mild however when the wind was not at northwest. He asserted that those who "seated themselves near great marshes whether in America or Europe are subject to agues but those who are planted more remote from marshes or standing water are exceedingly healthy." He enthusiastically cited a family of 12, not one of whom had been sick in 9 years. He declared the air was of so severe and excellent a temper that it gave a strong appetite and a quick digestion, that men found themselves more lightsome, more prone and more able to do all youthful exercises than in England, and the women were very fruitful and the children had fresh sanguine complexions.

But in the face of all this flattering description of a happy land

the assurance of summer fevers could not be overlooked. Governor Archdale in 1707 published "a New Description of the Fertile and Pleasant Province of Carolina" and had to admit that "gentle touches of the ague and fever in July and August were common." He defended the climate however by asserting that he had caught only one "careless violent cold" in five years. Those that suffered did so by "carelessness in their clothing or intemperence" and his Excellency the Governor warned "that such as every one sows, such shall he reap!"

Mr. Peter Purry in 1731 used the same argument saying that "if people are sick, 'tis generally an effect of their bad conduct, and not knowing how to regulate themselves suitably to the country." He noted as others had done that the new arrivals were more susceptible to ague and fever. Purry was more candid however than his predecessors and frankly admitted that there had been sickness and even the plague.

That sickness is a punishment for the sins of man is a very old idea. Almost equally ancient is the belief that disease, especially fevers were related to the weather. Hippocrates taught that climate had a marked influence in causing disease. He described three types of climate and the diseases peculiar to each. The air, by its hot moisture and impurities, and the wind by its force and direction were important factors. Sydenham in the 17th century speaks of the "atmospheric influence" during epidemics and the "secret inexplicable alteration of the air." Gradually the atmosphere itself was released from causal blame but it remained in some way a vehicle of disease.

An idea closely associated with the weather persisted throughout the 18th century, namely that the cause of disease was to be found in the foul odor that came from filth and decay. For example the yellow fever epidemic of 1793 in Philadelphia was caused by a ship load of coffee from Brazil that was allowed to rot at the wharves. This thesis was championed by such laymen as Noah Webster and medically it was sponsored by no less an authority than Benjamin Rush. Rush was extreme in his views. Infectious diseases of all kinds were caused by organic decay. He blamed drains, cesspools, privies, bilge water, decaying weeds and even rotting cabbage for the epidemics of fever. Decaying matter gave off a death dealing "something" but whether it was

solid particles, gases or some living organism no one had any clear opinion. At any rate organic decay occurred most rapidly in the summer when the temperatures were high, and here was the relation of climate and disease.

This belief in the meteorological origin or at least influence on some of the ills of mankind is thus easily understood. Malaria was the "mala aria" arising from the swamps. Diseases spread and lacked control like the air itself. Fevers sprang up and raged in the summer months and then subsided in the colder months only to be followed by respiratory complaints associated with another type of atmosphere.

Three epidemic diseases plagued Carolina; smallpox, malaria and yellow fever. From 1700 to 1802 when vaccination was introduced in Charleston there had been seven epidemics of smallpox. One of these in 1760 had a mortality of 1 in 4 for all afflicted, and a total of about 1000 died that year. Malaria was known as "country fever." It was rarely fatal and kept under control with Peruvian bark. It was yellow fever that ravaged the coastal cities. New comers were particularly susceptible and the disease was therefore often called "Stranger's fever." All who could on the first report of an outbreak, fled to the dry pinelands for the summer. Trade languished; ships stayed away and fear ruled the day. The first case in Charlestown seems to have been recognized on the 28th or 29th of August 1699. A letter from the Governor and Council describes it as a "most infectious, pestilential and mortal distemper" which was brought in from the Barbadoes or Providence. From its appearance in August until November there were 160 deaths. Epidemics occurred in 1703, 1728, 1732, 1739, 1745 and 1748. Sporadic cases were always present which kept the population in nervous dread. Curiously for 44 years after 1748 there were no epidemics, then in 1792 a series began which lasted for some ten years, nearly all of which were very severe.

It is interesting to note that outbreaks of yellow fever occurred as early as May and lasted until November, the height usually being in August and September. Children mostly escaped. Unseasoned Negroes were not exempt but had the disease in a milder form and were more generally cured. Persons from the West Indies also enjoyed a similar immunity. While the horror of the

Carolina epidemics has not been woven into literary recitals it was equal to anything experienced in Philadelphia.

Believing, as the general population did, that the various fevers were in some way related to the weather, it would be expected that someone would record the weather changes with some degree of care. The surprising thing is that no one did this, apparently in all America, and certainly not in Carolina before 1738. Governor James Glen in 1750 says that he had for some time kept a diary of the weather but it was evidently of little value for in his article on the climate of Carolina he only states that the thermometer had been known to rise to 98 degrees in summer and fall to 10 in winter. As for the rest he filled in with weird stories of two quart hot water bottles freezing in bed and a jar with a live eel being frozen to the bottom.

The one important point on Governor Glen's report was his statement that he had found "a very curious gentleman, one Doctor Lining" who had made observations on the weather and tabulated them. Thus after a long introduction I come to one of the important personages of this little essay. Surely a gentleman who in 1738 should begin accurate weather observations destined to last a dozen or more years, might at that day have been described as "curious" but that he also planned to find exactly what effect the weather had on the human body by quantitative experiments, stamps him as a really first-class scientist.

John Lining born in April 1708, was the eldest son and second child of the Reverend Thomas Lining and his wife Ann Hamilton who lived in Lanarkshire, Scotland. The Reverend Thomas was a man of some affairs and had served as burgess of Glasgow. On a salary of 125 bushels of oatmeal, 65 bushels of barley and 35 pounds sterling the Linings reared seven children. In 1730 John emigrated to Charlestown as a young doctor. No one has been able to find where he studied for he is not listed either at Glasgow or Edinburgh. However his success during the next thirty years proved he must have been well trained. He arrived between the two severe yellow fever epidemics of 1728 and 1732 and there is no doubt he had plenty to do.

By 1733 he was established as his own druggist, as was the custom among doctors of the period. On August 4th of that year the South Carolina Gazette carried an advertisement, "To be

sold by John Lining, Orange, Cinnamon, and Annisees Waters, etc and Spirits of Wine." In later advertisements prices were added. In one "a pine frame of a Dutch roof'd house" is offered for sale. Drug stores seem to have been about the same in all times. He became Senior Warden in the "Ancient and Accepted Order of Free Masons" of Charleston which was the first lodge organized in America.

In June 28, 1739 John Lining married a Sarah Hill, daughter of Charles Hill who owned a large plantation known as Hillsborough about 10 miles west of the city. Charles Hill was a man of means. He became Chief Justice of the Province and at his death in 1734 left a house on Broad and King Street which later became the home of the Linings. In this building which still stands were made the first weather observations with instruments in America.

In 1754 Dr. Lining's name is associated with a Dr. Chalmers and they "desire the Favor of all Persons indebted to us to pay their respective debts on or before the first of April next." In 1756 he offered the old home at Broad St. and King for sale and the family evidently moved to the Hillsborough plantation. That he was here in 1756 we know from his advertising fire wood for sale as well as offering a reward for the return of a colored man, George. In 1760 he evidently had returned to the city, for he advertises "Medicines in boxes with directions for their use in case of Pleurisies, Perepneumonies, Inflammation of the throat, continuous, nervous, intermitting and remitting fevers and fluxes. Price of each box twenty pounds."

We would like to think that he returned to the city to help in the epidemic of smallpox which was unusually severe in 1760. That year out of a population of 6,000, 730 died. The terror of the time was well expressed by some unhonored poet in the Gazette:

> Disease malignant fills the air
> Its noxious atoms far and near
> Spread through the ambient atmosphere:
> With doubtful flight and casual wing,
> Scattering their pestilential sting,
> Or thro' the pores or lungs they pass
> And taint your elemental mass.

"Dr. Lining survived the epidemic, to say nothing of the poetry, but in September of the year 1760 his death notice appeared.

"On Sunday last (September 21) died, very much lamented, John Lining, Esq., a gentleman eminent for his application and experience in discovering the causes, nature and cure of the disorders incident to this province, where he had practiced physic upwards of twenty years; and who possessed all the good qualifications that could render his loss great, as a physician, husband, father, master, friend, neighbor, companion, etc."

He left a widow and seven surviving children. His will was characteristic of his simplicity and directness. He merely said, "Into the Hands of God hoping for remission of my sins and a blessed resurrection to eternal life through the mercy of my Lord and Savior, Jesus Christ, my body I give to the grave and as for all my worldly estate I give it to my loving wife Sarah, knowing well her affection for our children, and that in due time she will make a suitable provision for them."

Let us now return to John Lining's scientific life. The letter of Governor Glen indicates that Lining had been keeping records of temperature and rainfall previous to 1738 although the known records begin on that date. These records, however, would probably never have been preserved had he not made further experiments "to discover the influence of our different seasons upon the humane bodie." These were statistical experiments made on himself and carried on throughout the year 1740 "with no small labour, confinement and expense in the loss of Practice." Even this added interest would probably not have saved them had not the author sent them with an explanatory letter to Charles Pinckney who was then in London and who later became the Chief Justice of our Supreme Court. Pinckney in turn forwarded them to James Jurin, Secretary of the Royal Society of London, who had them published in the Philosophical Transactions of the Royal Society, Vol. XLII, pages 491–509, 1742–43. Thus our Carolina doctor enters his hall of fame.

A photostatic copy of his four page letter is now available and I quote his opening paragraphs.

"That candid and generous Principle which so universally possesses the breasts of all true friends to physical literature, disposing them to give assistance and advice even to such of the

illiterates who show a disposition of enquiry after truth; and that eminent character you so justly bear in the learned world, were sufficient arguments with me to lay before you, as a specimen, one of my Metereo-Statical Tables. The favor of your opinion of the method I have observed will be most acceptable."

"What first induced me to enter upon this course was that I might experimentally discover the influences of our different seasons upon the human bodie, by which I might arrive at some more certain knowledge of the courses of our epidemic diseases, which as regularly return at their stated season as a good clock strikes 12 when the sun is in the meridian, and therefore must proceed from some general course operations uniformly in the returning different seasons."

"From the Histories of the Air and Epidemic Diseases, we learn what constitutions of the air are productive of certain diseases; were we however, well furnished with a course of Statical Experiments of one whole year, together with the Historie of the weather, we probably might have more distinct views of the nature of the diseases themselves, by knowing experimentally the changes produced on our constitutions, disposing us to such and such diseases in certain periods of the year."

The data that Lining put in his statical tables was really quite considerable. Each day he weighed himself twice, immediately after rising and just before going to bed at night. The day and night output of urine was carefully kept. The number of stools per day and their weight was diligently recorded. The loss by perspiration was computed from the preceding observations. The amount of food taken, fluid drunk and the type of exercise for the day is duly recorded. On the meteorological side there are complete records; temperature, humidity, precipitation, wind velocity, sky conditions and barometric readings. Even the occurrence of thunder was noted. When one thinks that these observations were made each day for a full year, one really wonders at the zeal and tirelessness of the man. In the face of it all he apologizes for his inability to make more studies on the urine, a situation he could not control because of lack of suitable apparatus.

The instruments he used were the following:

The barometer was a portable one, the diameter of its bore

being about 1/5 of an inch. He evidently had two thermometers. One was marked in Fahrenheits. The other was made by Thomas Heath in London and was divided into 90 equal parts, 65 being the freezing point. His hygroscope was a whip cord which had a total length variation of 5 inches. These instruments were placed in a box on the outside of a northeast window and protected from sun and rain but with good ventilation. He does not tell us his device for measuring precipitation. It was probably a simple open vessel of some kind. He had no areonometer but judged the force of the wind by his senses. He recognized the different degrees of velocity.

Lining's first letter to the Royal Society was dated January 22, 1741. The letter was in English script but the tables were all labeled in Latin. The year's observations began March 1, 1740, so they were not completed when this first letter was sent. On April 11, 1741, he sent a duplicate letter with the remaining tables.

In these two letters which evidently reached the Royal Society through the kindness of Pinckney, Lining does not indicate any conclusion at which he may have arrived from his experiments. In a letter to the Society under date of Jan. 29, 1743, however, he discloses his opinions. The deductions that he makes from his tables "appear to point out the Physical Principles from whence we may account for the production of these epidemic diseases of the different seasons which are not infections. For are not these the effects of different constitutions of the air on human bodies? And are not the increments and decrements of the sensible and insensible excretions, regard at the same time being had to the quantity and quality of the ingesta and to the exercise, etc., the only index of the changes produced on the human constitution by the vicissitudes of the weather. That indeed was the only view I had in going through these troublesome experiments with so great assiduity for one year. The design was benevolent and I am afraid far superior to my capacity especially as I am situated in a place where I can have no immediate assistance."

Although he showed by very carefully made quantitative measurements that as the daily temperatures rose in the "sickly months" of summer, the daily sensible perspiration increased and the diurnal urine decreased relative to the nocturnal, there

is nothing very exciting about such conclusions, however good the data may be. In no case so far as I can find did Lining try to correlate such changes with any of the symptoms seen in patients with the epidemic fevers.

By the end of 1748 Lining had had a great deal more experience with yellow fever and his previous idea that the epidemic fevers were not infectious was discarded. In a letter to Dr. Robert Whytt, Professor of Medicine in the University of Edinburgh, under date of December 14, 1753, he gives a detailed description of yellow fever as it appeared to him in the great epidemic of 1748. This most excellent and accurate account is sometimes claimed to be the first report of the disease to be sent from America. The claim is however doubtful, for Dr. John Mitchell of Virginia wrote a paper on yellow fever in 1744 which was sent to Benjamin Franklin to be read before the American Philosophical Society of Philadelphia. Mitchell's letter later came to the attention of Dr. Benjamin Rush and was one of the chief influences in forming Rush's opinions on yellow fever.

Of Dr. Lining's letter to Dr. Whytt we shall quote only the paragraph in which he renounces any ideas that the weather itself is etiologically responsible for yellow fever.

"This fever does not seem to take its origin from any particular constitution of the weather, independent of infectious miasmata, for within twenty-five years it has only been epidemic in 1732, 1739, 1745, and 1748, though none of these years were either warmer or more rainy, except 1739, than several other years in which we had not one incident of any one being seized with the fever. But that it is really an infectious disease seems plain, because almost all the nurses caught it and died of it, and as soon as it appeared in town it invaded newcomers, those who had never had it, and country people when they came to town, while those who remained in the country escaped it, as well as those who had had it, though they walked about the town, visited the sick in all the different stadia of the disease, and attended the funerals of those who died of it. And lastly the disease was always traced to some person who had lately arrived from some of the West Indian Islands. Although the infection was spread with some celerity through the town, yet if any from the country received it in town and sickened on their return home, the

infection spread no further not even so much as to one in the same house."

Having renounced the climate as being directly responsible for the epidemic fevers, Dr. Lining seems not to have speculated further on their causes. They were "infectious," of that he was sure. He nowhere expresses himself, but it is doubtful if he would have subscribed to any belief that the origin of yellow fever was local and due to emanation from animal and vegetable putrification. Opposed to any such idea is his observation that the epidemics always arose from a West Indian importation. Insects are never mentioned and we can be sure he never even dreamed of a mosquito vector.

It will be remembered that Lining's last letter to the Royal Society was dated in 1743. Ten years later, April 9, 1753, he again wrote that learned body, enclosing a table giving the rainfall in Charleston for a period of 15 years and remarking that if such a record were continued for half a century it might be of use in discovering the changes made in a climate by clearing the land of its woods.

Dr. Lining's interest in meteorology extended beyond recording weather observations for on May 15, 1753, the Gentleman's Magazine published a letter from the "Ingenious Dr. Lining of Charleston" which showed that he had taken up some of Franklin's electrical experiments for serious investigation. We quote from this letter.

"I have several times this season when there was an appearance of a thunderstorm, succeeded in making Mr. Franklin's experiment with a kite, for drawing the lightning from the clouds and last Monday repeated the same with remarkable success before many spectators. The flow of the electrical fluid, or the matter of lightning, was so rapid and copious down the line, near 700 feet long, to the key appended at the lower end of the line, that from thence I obtained sparks of lightning as thick and as long as the first two joints of a man's little finger, and these as quick one after another, as I could bring the loop of wire, which I used for that purpose within about two inches of the key; and the snapping from the key were so smart and loud that they were heard at the distance of at least 200 yards. A ten ounce phial coated was then properly suspended by the key that it might be

charged. . . . I then endeavored, without taking the phial off the key to discharge it in the normal manner, but as soon as I brought the loop of wire towards the coating of the phial, I received such a shock up to my shoulder that I failed in the attempt. . . ."

Just how Lining heard of Franklin's experiments is not known, but at any rate a correspondence developed between the two. A letter from Franklin in 1757 is extant in which he introduces a gentleman to the Doctor's kind attention. An attack of gout in 1754 seems to have curbed Doctor Lining's experiments with lightning and in his own words "perhaps prevented my meeting with the same unhappy fate with Professor Richman" who was killed by a discharge over his ungrounded apparatus.

John Lining was by no means the only doctor of Carolina who was a meteorologist. At one time he was in partnership with a Dr. Lionel Chalmers who also made weather observations. Dr. Chalmers seems to have been an independent observer and not merely influenced by his older friend and partner. His observations were made from 1750 to 1759 but they were not published until 1776. They then appeared in a book published in London under the title of "An Account of the Weather and Diseases of South Carolina." In his work he inserted some of Doctor Lining's tables to show how the secretions and excretions were affected by the weather, remarking that if they did not instruct, they might at least amuse some readers. Although Dr. Chalmers was an important medical man of the day he did not particularly advance our knowledge of the causes of the epidemic fevers.

Dr. Chalmers was born in 1715 in Cambelton, Scotland. In 1737 he came to Carolina and here he practiced for forty years. It is said he never affected any mystery in his practice, but employed the knowledge he had acquired for the good of mankind. He was reputed to be a loyalist but this is not entirely true. He died May 8, 1777.

Another doctor, George Milligan, at least deserves mention for his interest in weather and disease. In 1770 he published a "Short Description of the Province of South Carolina with an account of the Air, Weather and Diseases at Charleston." It was a description of general nature without any meteorological tables. Milligan himself ran into a good deal of trouble. He became

Chief Surgeon to his majesty's forces during the Revolution and finally had to flee the country. Arthur Middleton, one of the signers of the Declaration of Independence, refers to his flight in these words: "Probably he had an unconquerable dislike to the mode of clothing lately adopted in these scarce times (tar and feathers) and by no means wished to be exalted (hanged) in this damned hot country; but would rather have a high place in Scotland."

In the study of climate and disease the Medical Society of South Carolina also played its part. The Society was organized in 1789, not for the purpose of keeping weather records, to be sure, but this was a rather natural outgrowth of a common meteorological interest. The driving spirit along these lines seems to have been Dr. David Ramsay who was Treasurer and who in 1790 introduced a resolution that at every meeting the President should request the members to communicate information respecting the weather and the diseases of the previous month. Shortly thereafter in 1791 Doctor Ramsay was asked to take measures to get a barometer, thermometer and hygrometer. It was not until 1794 that three tin vessels were procured to serve as rain gauges. There is no evidence that Dr. Ramsay ever did any observing himself. This truly arduous task was taken on by Dr. Robert Wilson early in 1792. He did not lay the burden down until 1812, that is, for 20 years he kept accurate records of temperature, humidity, barometric pressure, rainfall, wind direction and velocities and the occurrence of thunder and lightning. Dr. Wilson relinquished his labors in 1812 and they were subsequently undertaken by a Dr. Joseph Johnson. If we feel that Dr. Wilson's 20-year task was Herculean, what shall we say about the endeavors of Dr. Johnson who kept the weather records until his death, 50 years later. This is probably the longest period of weather observations ever made by any one person.

A brief biographical account of these three meteorologically-minded men must suffice. Dr. David Ramsay was born in Lancaster County, Pennsylvania, on April 2, 1749. He is said to have read the Bible at 6 and to have read all the classics by the time he was 12. At 13 he was a tutor in a reputable Carlisle Academy. At 16 he took his B.A. degree at Princeton. He studied medicine with Dr. Bond in Philadelphia for two years and also

attended lectures at the College of Pennsylvania under Dr. Rush. On coming to Charleston in 1773 he brought a letter of recommendation with him from Dr. Rush which stated that he was "far superior to any person we have ever graduated at our college."

Dr. Robert Wilson was born at Ceres in Scotland in 1736. He was a graduate of Edinburgh. He practiced a year in Scotland, went up to London and then became interested in the Carolinas. He arrived in Charleston in 1752. During the Revolution he was an avowed loyalist and was one of those who signed a congratulatory letter to Cornwallis. So honest was he and so highly respected he did not lose prestige or fortune by this act or his beliefs. He died in 1815 at the age of 79.

Dr. Joseph Johnson was the only native American of the medical men mentioned, being born in the environs of Charleston in 1776. He was educated in the classics at the College of Charleston and took his M.D. degree at Pennsylvania in 1797, where he studied under Dr. Rush. He lived until the Civil War, dying in 1862 at the ripe age of 86.

In conclusion we may summarize the beliefs of these various men regarding yellow fever, as supported by their meteorological observations. John Lining came to the conclusion that yellow fever was not related to any constitution of the atmosphere, independent of infectious miasmata. It was definitely an infectious disease. It could always be traced to someone who brought it into the colony, usually from the constant source in the West Indies. It was not a contagion, for one might receive it in Charleston but go to his home in the country without giving it to anyone else in the family. Just how a West Indian with the fever could start up infectious miasmata in Charleston, but not in the pine lands west of the city, seems never to have presented itself to him as a medical problem.

Ramsay, Wilson and Johnson were all dominated by the opinions of Benjamin Rush. Not one of them had any critical ability. Rush believed that the summer fevers were all produced by miasmata or exhalations from decaying vegetable matter. It was always possible to find a local cause for every epidemic of yellow fever, and so Rush and his followers doubted that it could be imported. It was observed that the disease did not spread in the

country or in the hospitals when they were beyond the influence of impure air, that is, air that smelled of decaying animal and vegetable matter. It was known that it was not propagated by specific matter from the bodies of sick people or even by inoculation. All this confirmed their theories of infectious miasmata. It did lead, however, to better sanitation and thus helped reduce the incidence of the disease as we now know by destroying the life of the insect vector. The meteorological studies of these men were used to confirm the idea that heat, humidity, water, decomposition, and miasmata were factors in causing yellow fever. That frost and heavy rain, both beneficial, acted on the air and not the patient was also in their favor.

Here and there incidents occurred which led to doubt but they were brushed aside. An amusing one was the case of William Withers. In attempting to rob the South Carolina Bank he entered the sewer at Queen and Church streets and attempted to tunnel under the bank. He was underground for 90 days. On being discovered he was in rather a sorry plight but he did not have yellow fever as he really should have had. This aroused considerable discussion but it was finally decided that the great excitement of Wither's mind from the project of accumulating wealth must have counteracted the miasmata of the drains.

During all these years, the real offender, the anopheles mosquito reigned without the slightest suspicion being cast on his character. He was a nuisance but not a murderer. Only once was he even mentioned and curiously this was by Benjamin Rush in Philadelphia who said in one of the epidemics that the "muschetoes" were uncommonly numerous and a certain sign of an unwholesome atmosphere.

It is a melancholy conclusion merely to emphasize the inability of man to see the truth. After a hundred years of weather observations one wonders why some genius didn't associate clouds of mosquitoes with yellow fever as well as clouds of rain.

# ASSAY OF THYROTROPHIC ACTIVITY OF A PITUITARY TUMOR IN THYROPROTEIN-TREATED MICE*

DAVID M. MORRIS

The thyrotrophic activity of certain pituitary tumors induced in C57Bl mice by radiothyroidectomy has been studied by Burnett, Anderson, Gadsden, and Furth (1954), using various assay procedures. These include analysis of the morphological response of the thyroid, thyroidal $I^{131}$ retention, and total body retention of $I^{131}$ in mice and chicks following injection of the tumor material. The isotopic assay procedures proved to be convenient and sensitive, but response of the assay animal to a given dose of the tumor extract was variable. Despite this variability there was some indication that the homologous mouse was more sensitive than the chick to the pituitary tumor material, as judged by isotopic methods of assay. It seemed desirable, therefore, to try to increase the uniformity of response of the mouse in an isotopic method for assaying this tumor material. The present paper presents the results of a mouse assay of such material by a method in which the variability was markedly reduced.

It is well known that the response of the thyroid to thyrotrophin is enhanced by hypophysectomy. Since such an operation is difficult, chemical suppression of the pituitary of assay animals has been reorted to by several workers. Overbeck (1953) has used nonhypophysectomized rats given an iodinated casein ration in an assay based on thyroidal $I^{131}$ uptake. Gilliland and Strudwick (1953) have studied the effect of *L*-thyroxine in suppressing the chick pituitary, and Querido, Kassenaar, and Lameyer (1953) have studied the thyroidal $I^{131}$ uptake in iodocasein-fed mice in response to thyrotrophin injections. In the work presented here the mouse pituitary was suppressed with thyroprotein injections

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and the activity of thyrotrophic pituitary tumor extracts was measured by thyroidal $I^{131}$ uptake. In contrast with the saline-injected control mice given similar levels of tumor extract, thyroprotein-treated animals showed a marked increase in sensitivity and gave a more uniform response.

*Methods.* Mice (C57Bl males, weighing 24 to 33 g.) were injected daily for 3 days subcutaneously with either 0.2 ml. of thyroprotein solution[1] or 0.2 ml. saline. Twenty-four hours after the last injection, tumor extract was injected intraperitoneally, and 24 hours later, a tracer dose (5–15 $\mu$c) of carrier-free $I^{131}$ was injected intraperitoneally. The animals were killed with ether 4 hours later and a section of trachea with the thyroid attached was removed and placed in a scintillation counter to measure its activity. Uptake of radioiodine was expressed as percentage of the injected dose. The injected dose was at first measured by lowering each animal into an ionization chamber following the radioiodine injection, but in later experiments the activity of an aliquot of the injection solution was measured since this was found sufficiently accurate. The tumor was the dependent type, induced by the destruction of the thyroid gland in C57Bl mice by injection of radioiodine. Details of the development of such tumors are described by Furth and Burnett (1951). The material injected in the present study was prepared by lyophilization of a saline extract of tumor homogenate. The amounts mentioned in the following experiments refer to dry weights taken up in physiological saline. Similar material was shown in chick assays by Burnett *et al.* to contain 0.007–0.02 USP units of thyrotrophic hormone (TSH) per mg. of dry weight.

## *Results*

Data showing the results of 5 experiments in which were used several different dose levels of thyrotrophin tumor extract in saline-injected controls and in mice pretreated with thyroprotein are shown in table 1. The low activity of the thyroid of the thyroprotein-treated mice is apparent from the low $I^{131}$ uptake seen in the groups receiving no tumor material. The average mean value for percentage uptake in all the saline-injected controls was 6.29,

[1] Thyroprotein was kindly furnished by Parke, Davis & Co. and contained 0.00125 g/ml.

TABLE 1

*Effect of Pituitary Tumor Extract on $I^{131}$ Accumulation by Thyroids*

| Exp. No. | Tumor extract (mg) | Mean $I^{131}$ uptake in Saline treated mice (Per cent of inj. dose) | Mean $I^{131}$ uptake in Thyroprotein-treated mice (Per cent of inj. dose) |
|---|---|---|---|
| I | .0 | 4.33 | .66 |
| | .4 | 3.35 | 1.45 |
| | .8 | 4.17 | 3.16 |
| II | .0 | 3.32 | .24 |
| | .6 | 2.58 | 1.75 |
| | 1.2 | 4.37 | 2.07 |
| III | .0 | 11.22* | .... |
| | .1 | 13.92† | .... |
| | .2 | 9.10† | .... |
| | .4 | 9.98† | .... |
| | .8 | 17.33† | .... |
| IV | .0 | .... | 1.07 |
| | .1 | .... | 1.44 |
| | .2 | .... | 1.77 |
| V | .0 | .... | .66 |
| | .15 | .... | .98 |
| | .3 | .... | 1.73 |
| | .6 | .... | 2.67 |
| | 1.20 | .... | 6.86 |
| | 2.40 | .... | 12.77 |

* Two animals.
† Four animals. All other points represent 5 animals.

whereas that for the thyroprotein-injected controls was 0.66. The iodine accumulation was increased by very small amounts of injected tumor material in the thyroprotein-treated series but no real effect was noted in the saline-injected animals except perhaps after the largest dose of tumor extract. The graded response of the thyroid of the experimental series is also shown by the data in table 1 and in figure I which also emphasizes the difference in variability in the 2 series of animals. In this figure, for each series, straight lines having a common slope were fitted to each experiment by the method of least squares. The observed mean uptakes for each experiment were then adjusted to an average intercept and the two series then plotted. Confidence limits for each of the 2 curves have also been plotted.

The difference in the ability of the thyroid to take up injected radioiodine in the control and experimental series is evidenced by the difference in intercept of the 2 curves. This is a reflection of the expected suppression of the thyrotrophic activity of the animal's pituitary by the thyroprotein. A dose-response curve of

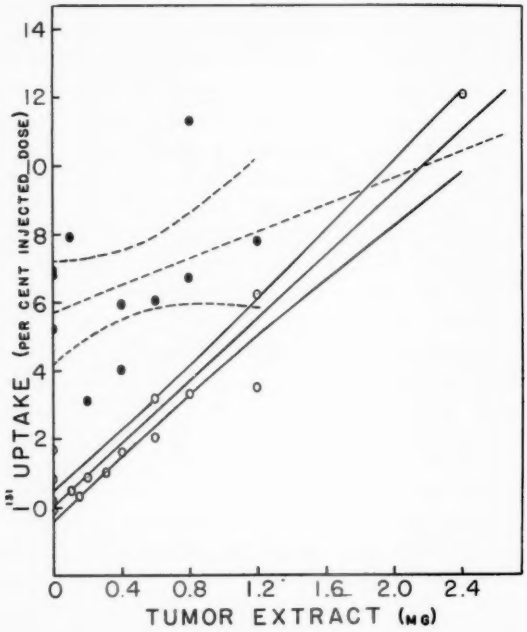


FIG. I. Relation of dose of tumor extract to accumulation of radio-iodine in thyroid of saline-injected and of thyroprotein-injected mice. Broken lines: curve fitted to saline-injected series shown by solid circles with calculated confidence limit at 95 per cent level. Solid lines: curve fitted to thyroprotein-injected series shown by open circles with confidence limit calculated at 95 per cent level.

significant slope was obtained only with the thyroprotein-treated series. The regression coefficient of the curve of the thyroprotein-treated mice was calculated as 4.5645 with a standard error of 0.3070. Thus the slope differs from zero at a level of significance less than 1 per cent. The regression coefficient of the curve of the control series equals 1.9421 with a standard error of 1.290, so that this slope does not differ significantly from zero. It was calculated that with 9 thyroprotein-treated animals in a group one could detect a response to 0.4 mg. of the tumor extract at the 5 per cent level with 95 per cent assurance. Higher doses could be detected

with fewer mice (*e.g.*, 0.7 mg. would give a significant response with as few as 4 animals). Relatively little variability was seen in the thyroprotein-treated series as compared with a very marked variability in the saline-injected control mice.

It is clear that the method used here for assay of pituitary tumor extract obviated some of the difficulties encountered in other methods used by Burnett *et al.*, and this would suggest that the method might have value in other applications. However, preliminary experiments in which USP standard was used to prepare a dose response curve by this procedure have shown unexpected variability at some dose levels. With 0.025 USP units the error is about 20 per cent. This discrepancy is difficult to explain unless it may be due to a different strain of mouse (C3H) used in the USP experiment. It may be attributed, at least in part, to the greater specificity of response of the mouse to homologous material. Greater sensitivity and uniformity might be achieved by altering the method somewhat; for example, by giving the thyrotrophin in divided doses and increasing the time between $I^{131}$ injection and autopsy. After the completion of the present work Querido *et al.* (1953) reported some success with a similar procedure using iodocasein. These workers suppressed the pituitary of mice by feeding iodocasein prior to and during injection of TSH. The hormone was given in three divided doses and a tracer dose of $I^{131}$ was injected along with the last TSH injection. Twenty-four hours hours later the radioactivity of the thyroids was determined. They report a sensitivity to as little as 10 $\mu$g (0.0005 units) USP standard and significant changes as a result of injection of 0.012 USP units.

*Summary*. Extracts of a thyrotrophic hormone-producing pituitary tumor have been assayed in mice, thyroidal $I^{131}$ uptake being used as a criterion of thyrotrophic potency. It is shown that pretreatment of the assay mouse with thyroprotein injections suppresses the animal's endogenous thyrotrophic hormone production and yields an animal which is markedly more sensitive and more uniformly reactive to this hormone administered in the form of tumor extract than the normal mouse.

*Acknowledgments*

The author is grateful to Dr. Jacob Furth for his aid and advice during the course of this work and for supplying the tumor material. Thanks are also due to Dr. Allyn W. Kimball for the statistical analysis.

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# EARLY DIAGNOSIS OF LEPTOSPIROSIS BY LYSIS-TEST WITH URINE-FRACTIONS*

EUGENE L. NOWICKI

The experience and the thorough knowledge of Dr. A. Packchanian in the field of leptospiroses, as well as the equipment and the stock cultures of his laboratory enabled a study of early diagnosis by new methods. These include lysis tests with urinary fractions.

Human and animal leptospiral infections have received during recent years an increasing attention because of their worldwide distribution in human beings as well as in animals. Within the last decade there has been remarkable change in the opinion of those familiar with leptospiral infections with regard to the importance of these diseases in human and veterinary medicine. The leptospiroses are now viewed as a group of diseases which may constitute a major public health problem. From the military standpoint, prevention and diagnosis of leptospirosis are of considerable significance, because military operations are usually carried out in areas exposed to the infection.

The diagnosis of leptospirosis has greatly progressed during recent years. Cultural methods, animal inoculation, the agglutination-lysis test, and complement-fixation assure the correct diagnosis of leptospirosis in humans and animals. The agglutination-lysis test, as introduced by Schüffner, or in Kahn tubes as used by Gochenour in the U. S. Army Medical Service Graduate School, demonstrates the antibodies in average on the twelfth day of infection with maximum titers obtained in the second and third weeks of illness. Leptospiral agglutinating antibodies persist at detectable levels in some instances as long as 500 days. "It is evident, therefore, that the examination of a single serum specimen is inadequate for the diagnosis of leptospirosis, rather a significant rise in the titers of serial serum specimens must be demonstrated" (Gochenour, 1953).

Complement-fixation is employed as Standard Kolmer quali-

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tative technics, first with undiluted serum, then in dilutions in positive cases. Gochenour is using a battery of three antigens prepared from *Leptospira icterohemorrhagiae*, *L. grippotyphosa*, and *L. hyos* in order to detect the leptospiral antibodies regardless of the infecting strain. On the average the test becomes positive on the tenth day of disease. The complement-fixing antibodies are ordinarily no longer demonstrable after the third month of illness.

The serological classification of pathogenic leptospires is closely connected with the serology and cultural diagnosis of leptospirosis. The methods of serologic typing and the grouping of leptospires are indicated by many investigators (Borg-Petersen, Collier, Gochenour, Gsell, Mochtar, Schüffner, v. Tiel, Walch-Sorgdrager, Wolff, Yamamoto, and others). Using the agglutination-lysis test and the cross-absorbtion test many leptospira strains have been identified (in Wolff's scheme 36 strains, Wolff, 1953), and the serological relations between those strains established.

The present status of laboratory diagnosis of leptospirosis can be reassumed as follows: the clinician can expect little help from the bacteriologist during the first week of the disease, except in the few cases where leptospires can be demonstrated microscopically in the blood of the patient. The effect of some drugs is assured only if therapy started between the first and fourth days of illness. The diagnostic methods of leptospiroses are too complicated to be used in laboratories which are not specialized in leptospires. It seems, therefore, of interest to try a new method of diagnosis of leptospires.

Several years ago clinical and laboratory experiments of specific agent in the urine (Nowicki, 1942) were reported. The method was worked out on the basis of the fact that injured organs produce proteolytic agents in the urine. These agents from urine, when incubated with different organs, react primarily with the organ which is injured. This reaction, which is very similar to serological reactions, shows the pathological conditions and degree of injury of the organs. The reaction is simple and, if accompanied by adequate controls, gives quantitative results. The experiments on patients undergoing X-ray treatment showed the degree of injury produced by irradiation (Nowicki,

1942). The test applied to the infectious diseases (typhoid) shows the condition of different organs during the illness. Changes in these conditions give a positive proteolytic test several days before they can be recorded by the usual clinical methods (Nowicki, 1943). In organic illnesses the test shows the intensity of pathologic changes and the response to the treatment. Immunological studies on specific agent in immunized rabbits, by using ovalbumin as antigen, have shown that the mentioned reaction manifests itself earlier than the antibody response, and that this reaction is specific. The agent appears for the first time in urine about 20 hours after antigen injection; it has a different activity (intensity)—depending on the method of antigen injections—during several days, and disappears when the precipitins in the serum reach a higher titer.

These previously reported experiments have led to the idea, that leptospira may act as an antigen for the production of a specific agent in the infected body, and that this agent could be isolated from urine. It was expected that the anti-leptospira agent would act as a lysin because lysis has been observed pre viously on the agglutination-lysis test. On the basis of this theoretical considerations the following experiments were carried out.

### *Lysins in the Urine of Rabbits Immunized Against Leptospira*

*Method of lysin isolation.* Several rabbits were immunized with Leptospira pyrogenes Salinem. The first five intravenous injections with killed leptospira were followed by injections with leptospira alive. The rabbits were kept in metabolism cages and the urine was collected over-night. First two samples were collected prior to inoculation for control. Each urine sample was treated in the following way:

1) Filtration through filter-paper if urine is turbid.
2) The pH adjusted to 7.0.
3) Filtration.
4) Note the amount of used urine.
5) Acetone added in equal parts and shaken.
6) Centrifuged at 1500 rpm for 5 minutes.
7) Supernate is discarded.
8) The tubes placed for several minutes on a filter paper in reversed position to discard the remained drops of the supernate.
9) The sediment redissolved in a tenth part of saline (for example 5 ml. saline for the sediment from 50 ml. of urine). Saline was added in a small amount (about 0.5 ml.), and the sediment mixed with a glass rod or a wooden applicator. The rest of saline was added and the tube shaken to obtain a homogenous suspension.

10) The suspension centrifuged and the supernate fluid retained.
11) Two drops of 1 per cent solution of Chinosol added to each ml. of suspension. The preserved suspension can be kept in ice-box for several days or even weeks in temperature between 2 and 4° C.
12) Before using the suspension, which was kept in the ice-box for several days, the pH is checked again and, if necessary, adjusted to pH 7.0.

*The lysis-test.* The lysis-test with the immunized rabbits was performed as follows: in a small test-tube 0.3 ml. of the suspension (urine fraction) was mixed with 0.3 ml. of dense leptospira culture in liquid medium (in this particular case Leptospira pyrogenes Salinem). The tubes with this mixture were plugged with corks and kept over night at 30° C. This was accompanied by two controls, namely normal rabbit urine with leptospires, and leptospira culture with saline.

The tests were read the next day: the liquid in each tube was mixed with a small pipette, and a drop was placed on a slide under a small cover-slip. The drop was observed in the dark field under dry objective 45 x. First the density of leptospires was checked in the controls. Then the density of leptospires in the other drops was checked and compared with controls. If the lysis was complete, namely no leptospira were seen in the drop, the lysis was evaluated as + + + + (4 plus); when few leptospires were present: + + +; with about 10–30 per cent leptospires left: + +, and when 30–60 per cent leptospires were left: +. If the density of leptospires was about the same as in the controls, the result was evaluated as negative (0).

*The lysins during immunization.* The lysins in the urine of immunized rabbits appear the second day after injection and can be detected by lysis-test during several days. The following injections of antigen (leptospira) produce some inhibition in the lysins production, but only for one day after each injection. The lysins decrease after the agglutinins titer becomes higher and they disappear almost completely after 14–16 days. The mentioned data are presented in figure I.

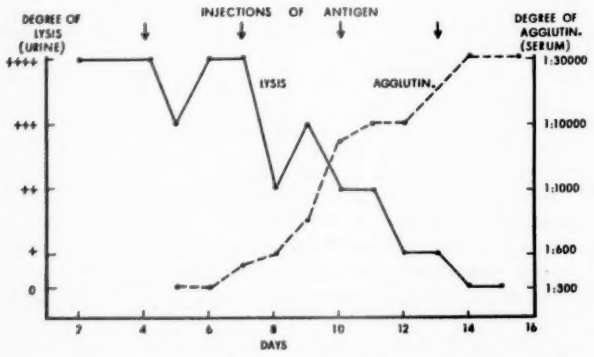


FIG. I. Lysins in the urine and agglutinins in the blood of rabbits immunized against Leptospira.

*The specificity of lysins.* After the presence of specific lysins against the strain used as antigen was established, the same lysins-suspension was used for the lysis-test with other strains of leptospires. The test was performed exactly in the same way as mentioned before. The results are presented in table 1. Urines from non-infected rabbits were used as controls. Table 1 shows

TABLE 1

*The Specificity of the Enzyme from Leptospira Pyrogenes in Lysis-Tests with Different Strains of Leptospires*

| Strain No. | Name | Received from | Lysis | Control | Remarks |
|---|---|---|---|---|---|
| 202 | L. autumnalis AB | Amsterdam | ++ | 0 | ..... |
| 203 | L. djassimani | Amsterdam | ++++ | 0 | ..... |
| 204 | L. hebdomadis | Amsterdam | ++ | 0 | ..... |
| 205 | L. icterohemorrhagiae AB | Amsterdam | ++++ | 0 | ..... |
| 206 | L. pomona | Amsterdam | ++++ | 0 | see 226 |
| 207 | L. pyrogenes Salinem | Amsterdam | ++++ | 0 | see 229 |
| 208 | L. semaranga Rat S | Amsterdam | 0 | 0 | ..... |
| 209 | L. australis A | Tokyo | ++++ | 0 | see 210 |
| 210 | L. australis A Ballico | Tokyo | ++++ | 0 | see 209 |
| 211 | L. autumnalis A | Tokyo | ++++ | 0 | ..... |
| 212 | L. benjamini | Tokyo | ++ | 0 | ..... |
| 213 | L. andaman A | Tokyo | 0 | 0 | ..... |
| 214 | L. ndambari | Tokyo | 0(+) | 0 | ..... |
| 215 | L. Cataviae | Tokyo | ++++ | 0 | ..... |
| 216 | L. schüffneri, Vl. 90C | Tokyo | 0 | 0 | see 221 |
| 217 | L. suis S.R. | Tokyo | +++ | 0 | ..... |
| 218 | L. canicola | Amsterdam | ++++ | 0 | see 223 |
| 219 | L. bataviae v. Tienen | Amsterdam | 0 | 0 | ..... |
| 221 | L. schüffneri Vl. 90C | Amsterdam | 0 | 0 | see 216 |
| 222 | L. hardjoprajitno | Tokyo | 0 | 0 | ..... |
| 223 | L. canicola | Tokyo | 0 | 0 | see 218 |
| 225 | L. naami | Tokyo | 0 | 0 | ..... |
| 226 | L. pomona | Tokyo | ++ | 0 | see 206 |
| 229 | L. pyrogenes | Tokyo | 0 | 0 | see 207 |
| 231 | L. sentoti Sentot | Tokyo | 0 | 0 | ..... |

the group specificity of lysins. In comparing this result with the sereological grouping of leptospires (Wolff, 1953) some difference is clearly seen. The specificity of lysins seems to differ from the specificity of agglutinins. The table shows the difference in lysins between some strains of identical species received from different laboratories (L. pyrogenes 207 and 229; L. canicola 218 and 223). Other strains of identical species and received from different places are identical in their lysis-reaction (L. schüffneri Vl.90C–217 and 221; L. australis A 209 and 210; L. pomona 206 and 226).

The group specificity of lysins seems to be wider than the group-specificity of agglutinins. Should this observation be confirmed by further experiments, it would facilitate the diagnosis of leptospirosis by using the lysis-test.

*Lysins in the Urine of a Pig Infected with Virulent Leptospira*

To follow the lysins in the urine during an infection and to evaluate the

clinical use of the lysis-test, a young pig was infected with a virulent strain of *Leptospira pomona* freshly isolated from a case of leptospirosis of swine.

TABLE 2

*Agglutination (Schüffner)-Test with Leptospira Pomona*

| Dilution of Serum | Degree of Agglutination: Pig Serum 6 hrs | Pig Serum 24 hrs | Normal Rabbit Serum 6 hrs | Normal Rabbit Serum 24 hrs |
|---|---|---|---|---|
| 1:30 | 0 | + | 0 | + |
| 1:100 | 0 | 0 | 0 | 0 |
| 1:300 | 0 | 0 | 0 | 0 |
| 1:1000 | 0 | 0 | 0 | 0 |
| 1:10000 | 0 | 0 | 0 | 0 |
| antigen control | 0 | 0 | 0 | 0 |

TABLE 3

*The Lysis-Test with Pig Urine Before Inoculation*

| Strain (antigen) | Degree of Lysis 4 hrs | 20 hrs |
|---|---|---|
| L. pomona | 0 | 0 |
| L. canicola | 0 | 0 |
| L. bataviae | 0 | 0 |
| L. icterohemorrhagiae | 0 | 0 |

The blood of the pig was tested for agglutinins and the urine examined by lysis-test before inoculation of leptospira. The results are presented in tables 2 and 3. After the lysis-test was negative with urine collected during three days, the pig was infected with the virulent strain of Leptospira pomona. The urine was then collected daily and blood every third day during 16 following days. The lysis-test presented in table 4 shows an important difference comparing with the lysis during the immunization of the rabbit (Fig. 1): in sixteen tests of table 4 none show complete lysis. Clinically the pig has not shown any symptoms of an acute illness. The appetite was good, temperature normal, no vomiting or diarrhea, no jaundice. The incomplete lysis is probably due to the mild clinical picture.

*The modified lysis-test for subclinical infections.* In order to obtain better results of the lysis-test for such mild infections the technic has been modified as follows: the antigen (leptospira culture) was centrifuged for 5 minutes at 1500 rpm. The supernate was pipetted off in a sterile tube and mixed with the pipette to obtain a homogenous suspension. Further, the antigen was pipetted into a small tube (0.3 ml.) and urine fraction added. Consequently, each tube contained an equal density of leptospira suspension. After incubation at 30° C. for about 16 hours the content in each tube was mixed with a separate pipette, and one drop used for microscopic examination. A small square was marked with pencil on the eyepiece lense. The leptospires were

TABLE 4

*The Lysis-Tests with Pig Urines After Infection*

| Day after Infection | Degree of Lysis | Day after Infection | Degree of Lysis |
|---|---|---|---|
| 2nd | +++ | 10th | +++ |
| 3rd | + | 11th | ++ |
| 4th | + | 12th | + |
| 5th | ++ | 13th | 0 |
| 6th | 0 | 14th | 0 |
| 7th | +++ | 15th | 0 |
| 8th | 0 | 16th | 0 |
| 9th | +++ | | |

TABLE 5

*Percentage of Lysis. Urines of Infected Pig*

| Controls | Urines (days) | Leptospires in 10 Squares | Approximate Percentage of Lysis |
|---|---|---|---|
| Antigen+saline | 0 | 21 | 0 |
| Antig.+norm. ur. | 0 | 23 | 0 |
| | 2nd | 2 | 90 |
| | 3rd | 12 | 50 |
| | 4th | 10 | 50 |
| | 5th | 6 | 70 |
| | 6th | 19 | 8 |
| | 7th | 3 | 85 |
| | 8th | 16 | 7 |
| | 9th | 2 | 90 |
| | 10th | 2 | 90 |
| | 11th | 5 | 75 |
| | 12th | 14 | 30 |
| | 13th | 20 | 0 |
| | 14th | 22 | 0 |
| | 15th | 19 | 1 |
| | 16th | 21 | 0 |

counted in 10 squares in each drop. While the lysis-test with 16 urine samples was performed on the same day with the same antigen, the degree of lysis was calculated in percentage as compared with controls. The table 5 and figure II show that the lysins appear on the second day of infection, and, except for some decline on the 6th and 8th days, they can be detected until the 12th day of infection. At that time the agglutinins in the blood are increasing to their maximum, and the lysins are disappearing. The picture is very similar to that presented in the figure I, except for the agglutinins titer, which in this case is much lower. This low agglutination titer is another sign of a mild infection in this particular pig.

*Lysins in the Urine of Patients Suspected for Weil's Disease*

The Laboratory of Microbiology is receiving blood specimens for routine

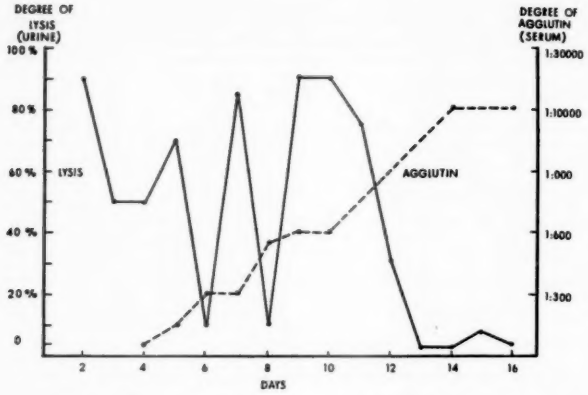


FIG. II. Lysins in the urine and agglutinins in the blood of a pig infected with Leptospira pomona.

diagnosis of Weil's disease from John Sealy Hospital in Galveston, as well as from other places in Texas. From some of these patients urine was obtained and the lysis-test performed. Two following examples are presented:

Patient No. 1: Hospital No. BA 8701E. First blood sample, and the urine for lysis test were received the second day of admission to the hospital. The patient had some jaundice and other clinical symptoms of Weil's disease. The results of the agglutination test and the lysis-test with urine fraction are presented in tables 6 and 7. Two days later the second sample of blood and

TABLE 6

*The Agglutination (Schüffner)-Test with Serum of Patient No. 1*

| Antigen used | Dilution of Serum | Degree of Agglutination | Degree of Lysis |
|---|---|---|---|
| L. icterohemorrhagiae | 1:10 | ++++ | 0 |
| | 1:30 | ++++ | 0 |
| | 1:100 | ++++ | ++ |
| | 1:300 | +++ | + |
| | 1:600 | + | + |
| | 1:1000 | 0 | 0 |
| | antigen control | 0 | 0 |
| L. canicola | 1:10 | ++++ | 0 |
| | 1:30 | ++++ | 0 |
| | 1:100 | +++ | 0 |
| | 1:300 | ++ | 0 |
| | 1:600 | 0 | 0 |
| | 1:1000 | 0 | 0 |
| | antigen control | 0 | 0 |

TABLE 7

*The Lysis-Test with Patient Urine (Technic as Described Before)*

| Strain | Degree of Lysis |
|---|---|
| L. icterohemorrhagiae | ++++ |
| L. canicola | +++ |
| L. pomona | + |

urine were received. The agglutination (Schüffner) test has shown an increase of titer up to 1:1000. The lysis-test with urine was as before: L. icterohemorrhagiae + + + +; L. canicola + + +; L. pomona +.

Patient No. 2: Hospital No. UA 013358, clinically suspected for Weil's disease. On the second day after admission the serum showed the agglutination titer 1:300 with L. icterohemorrhagiae. The urine of the patient used for the lysis test gave positive result with L. icterohemorrhagiae and L. canicola (Table 8). Because of the difference in the results between the agglu-

TABLE 8

*The Lysis-Test with Urine of Patient No. 2*

| Strain | Degree of Lysis |
|---|---|
| L. icterohemorrhagiae | ++++ |
| L. hebdomadis | 0 |
| L. canicola | ++++ |
| L. pomona | + |
| L. icterohemorrhagiae with normal urine (control) | 0 |

tination and lysis test the urine of the patient was centrifuged on the high speed centrifuge, and the sediment, redissolved in 1/10 of saline, injected into two *Peromyscus maniculatus gambeli* (1 ml. each). The tail-blood was examined every day for nine days. No leptospira was seen in the blood. The mice were then bled from the heart and the agglutination test performed. The agglutination titer of both mice was: with *L. canicola* 1:1000+, and with *L. icterohemorrhagie* 1:1000+.

*Preservation of Leptospira for the Lysis Test*

In order not to depend from cultures of leptospira, Chinosol (Potassium ortho-oxyquinoleine sulfate, Sandoz, Bale, Switzerland) was used as preservative for the antigen for the lysis-test. This compound was chosen because of its usefulness for preservation of urine, and non-interference with the lytic action. The results are presented in table 9. The most favorable concentration of Chinosol is 0.2 ml. of a 0.1 per cent solution per ml. of culture. The next step was to compare the best concentration of Chinosol with the routinely used concentration of formaldehyde (one drop of 1:10 solution for 5 ml. of culture). As table 10 shows, Chinosol is preserving leptospira in culture much

TABLE 9

*Preservation of Leptospires with Chinosol*

| Strain | Chinosol concentration per ml. of culture | Results (room temperature) 4 hrs | 24 hrs | 56 hrs | 4 days | 7 days |
|---|---|---|---|---|---|---|
| L. pomona | 0.2 ml. of a 1.0% sol. | killed | killed | deform. | | |
| | 0.1 ml. of a 1.0% sol. | killed | killed | deform. | | |
| | 0.2 ml. of a 0.1% sol. | alive | killed | same | no clumbs no lysis | no clumbs no lysis |
| | 0.15 ml. of a 0.1% sol. | alive | killed | same | no clumbs no lysis | no clumbs some lysis |
| | 0.05 ml. of a 0.1% sol. | alive | alive | contam. | | |
| L. australis | 0.2 ml. of a 1.0% sol. | killed | killed | deform. | | |
| | 0.12 ml. of a 1.0% sol. | killed | killed | deform. | | |
| | 0.2 ml. of a 0.12 ml. of a 0.1% sol. | alive | killed | killed lysis | no lysis | no clumbs lysis ab. 10% |
| | 0.05 ml. of a 0.1% sol. | alive | alive | contam. | | |

TABLE 10

*Chinosol and Formaldehyd Compared as Preservatives*

| Strain | Time (room temp.) | Chinosol 0.2 ml. of 0.1 Per cent Sol. per ml. of Culture | Formaldehyd 0.05 ml. of Sol. 1:50 per ml. of Culture |
|---|---|---|---|
| L. djassimani | 16 hrs. | killed no clumbs | killed some clumbs |
| | 3 days | no clumbs no lysis | some clumbs some lysis |
| | 7 days | no clumbs no lysis | clumbs lysis |
| L. hebdomadis | 16 hrs. | killed no clumbs | killed some clumbs |
| | 3 days | no clumbs no lysis | some clumbs some lysis |
| | 7 days | no clumbs no lysis | clumbs lysis |

better than formaldehyde if the antigen has to be used several days after preservation. The lysis-test performed with live leptospira and leptospira killed by Chinosol does not show any difference in results.

*Discussions.* The above experiments show that the human, as well as the animal body produce specific lytic agent in response to leptospiral infection. The agents are group-specific, and they

are eliminated in urine within about 20–30 hours after infection during 10–12 subsequent days. The agent can be detected by an easy lysis-test. The activity of the agent in the urine is not constant, thereby, for a correct diagnosis, urine samples from two following days must be used. Both urine samples, neutralized, filtered, and treated with acetone should be used for the lysis-test at the same time, with the same antigen in order to compare them correctly with the controls (antigen with saline and with normal urine) by counting the leptospires in a square marked on the ocular.

An important problem is to define the specificity of the lysis-test for the leptospira groups, as well as for other non-leptospiral infections. If successful, the lysis-test can prove a useful method of diagnosis of leptospirosis for the following reasons: 1) the lysis-test can detect the leptospira infection early enough to apply successful treatment; 2) it can be performed in any laboratory or even in a consultation office, providing the antigen is available.

From the theoretical point of view it is remarkable that the lysins differ in their nature from the agglutinins since the lysins are decreasing while the agglutinins are increasing in titer.

The clinical manifested leptospirosis, as well as active immunization, are producing lysins strong enough to manifest themselves through a complete lysis of leptospires in vitro. A sub-clinical infection produces less active lysins with the partial lysis in the lysis-test.

For a clinician, as well as for a epidemiologist the lysins present a great advantage because of their fast elimination from the blood stream. Hence, the active infection can be easily differentiated from cases with the positive serum agglutination which remains after a previous illness.

*Summary.* It has been demonstrated that the urine of a leptospira infected organism, human and animal, contains lytic agent specific for leptospira. These agents can be easily isolated from the urine, concentrated ten times and used for a lysis-test *in vitro*. The test becomes positive as early as 20–30 hours after infection. Leptospira culture, alive or preserved with Chinosol, serves as antigen. In cases of the clinical manifested illness, as well as during immunization, the lysis is complete with the anologous

strain and partial or negative with other strains (group specificity of lysins). During subclinical infections the lysis is only partial, and can be detected through counting of leptospires in several squares marked on the eyepiece of the microscope. The results of counting are then compared with the controls. The lysis-test has the following advantages: 1. easy technic; 2. early application, and 3. easy differentiation between the active infection and the serum positive cases without an active infection.

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# FRENCH CONTRIBUTIONS TO PHYSIOLOGY IN THE XIX CENTURY

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Much of our present day newspaper space is devoted to the attitude of France toward the re-arming of Germany, but there are fields other than military where rivalry between these two countries has been evident. Although one may emphasize the predominance of German influence on physiology in the latter part of the 19th century, it must be admitted that for at least the first half of this period France was in the lead.

The 19th century opened auspiciously in France for the development of physiology. Only a short time before falling a victim to the guillotine, Antoine Lavoisier had so transformed chemistry that its advance was to be both sure and rapid, and at the same time he showed how essential chemistry is for an understanding of the functioning of living organisms. His work on respiration had laid a solid foundation for the idea that the phenomena of life are not unique, nor are they governed by a special vital force, which had long been the accepted doctrine. Indeed, the supernatural character of the supposed force governing living action was implied in the name given to it, in the 18th century, "la force hyperméchanique." Lavoisier had shown that one of the most characteristic features of life, breathing, involved the use of the gas to which he had given the name oxygen, in a manner similar to its use in the burning of a candle. He concluded that the food a living creature eats furnishes carbon as a fuel, that the combustion of this fuel takes place during respiration, and that this combustion is the source of animal heat. Thus he laid the foundation for our modern ideas of metabolism, and its measurement.

The underlying principle, however, was not immediately accepted, and the older view that vital phenomena were in a class by themselves continued to be dominant, particularly in medi-

* From the Physiology Laboratory of the University of California, Berkeley. Presented at the meeting of the American Association for the Advancement of Science, December 30, 1954.

cine, until nearly the middle of the century. The study of physiology of course had its origin in medicine for which, at the beginning of the 19th century anatomy was considered the basic science. It seemed self-evident that function should be dependent on form, so that when, just after the Revolution, there was a thorough reorganization of medical education in France, provision was made for the Faculties of Medicine in the three cities, Paris, Montpellier, and Strasbourg, to have a chair which combined the teaching of anatomy and physiology. François Chaussier, who had drawn up the scheme, was chosen as the first incumbent of such a chair in Paris. When in the first year of the new century he withdrew from the professorship, a competition was held to fill the vacancy, and the brilliant young Bichat, who was then just thirty years old, presented himself as a candidate. The chair was, however, given to Duméril, and when in 1823 physiology was separated from anatomy at the University of Paris, Duméril became the first occupant of an independent chair of physiology in any university. Nevertheless, Bichat's influence on the science of physiology was far greater than that of Duméril, and indeed the future of physiological thought in Paris, and hence in France—for during the 19th century Paris quite overshadowed the provincial universities, even its ancient rival Montpellier—did not rest so much with the Faculty of Medicine as with those perhaps more independently-minded scholars who were freed from the task of teaching set courses, and lectured mainly on the results of their own researches. Although other names must be mentioned in the story of French physiology in the 19th century, François Magendie, Claude Bernard and Charles-Edouard Brown-Séquard, who occupied in turn the chair of Medicine at the Collège de France, may be said to dominate. Note that the title of their chair was not Physiology, but Medicine, and it would seem that Bernard's use of the term "experimental medicine"indicated that he considered it virtually synonomous with "experimental physiology."

In spite of Bichat's failure to obtain the chair of Anatomy and Physiology in the Paris Faculty of Medicine, his theories were in agreement with the prevailing medical ideas and were widely accepted and taught. He was unfortunately destined to live only two years into the new century, but so clearly and neatly were

his views expressed in his three published books, that for the next decade they were vigorously defended by a host of his admirers. He is often called the father of histology, though he distrusted and refused to use the instrument which we would now consider indispensable to such study, the microscope. His basic idea was that animals are an assemblage of organs, each organ performing its own special function, and that these organs, like chemical compounds—here he actually borrowed his terms from the new chemistry—are capable of being resolved into elements. These living elements are the tissues which he classified under 21 types, nervous, muscular, bony, etc. He ascribed to them four properties; two were physical, *i.e.*, elasticity and extensibility; the two others were vital, *i.e.*, sensitivity and contractility. For him there was always in life a conflict between the vital forces and the inanimate forces of physics and chemistry, the latter assuming full sway after death. This idea is embodied in his famous definition of life as "the sum total of those forces which resist death."

Bichat and particularly his followers therefore raised objections as to the validity of experiments on living things. For them the emphasis was on the instability of living phenomena, and Bichat himself had stated that it was futile for Lavoisier to have tried to calculate the quantity of air entering the living lung. His reason for this statement was, in his own words, "The instability of the vital forces marks all vital phenomena with an irregularity which distinguishes them from physical phenomena, the latter being remarkable for their stability."

Just as the first decade of the century was drawing to a close a protesting voice was heard. François Magendie had just received his M.D. degree and had been elected to membership in a scientific society La Société d'Émulation, one of whose founders had been Bichat, and whose Bulletin was claimed to be devoted to the preservation of his memory. Magendie's article, entitled "Some general ideas on the phenomena peculiar to living bodies," appeared in this Bulletin in 1809, and is was said that the young surgeon had made his "scientific debut" by a direct attack on Bichat. Realizing that living phenomena were definitely tied in with the new chemical concepts, he dared predict "Whenever the vital force animates a body of a given organization it will

produce given phenomena." In other words, physiological investigation under constant conditions should give as satisfactory results as investigation of non-living matter. This idea was developed and elaborated by August Comte in his system of philosophy, positivism, which so interested Magendie's pupil, Bernard, that he left a thick notebook filled with a résumé of and comments on Comte's lectures.

Experimental physiology was soon the order of the day, and although articles describing physiological experiments appeared in the records of various scientific societies and in independent pamphlets, such as Legallois' little book of 1812 in which he cited experiments showing that the respiratory center lies in the medulla of the brain, it was not until 1821 that the first magazine entirely devoted to experimental physiology appeared. This was Magendie's *Journal de Physiologie Expérimentale* whose early issues were mostly taken up with accounts of the editor's own work. Since so much of his interest at that time was centered about the action of drugs, such as strychnine and tartar emetic, the administration of which often lead to death, he added the words "et Pathologie" to the title of his journal in its second year. Strictly speaking, Magendie's career began in the field of experimental pharmacology. He is sometimes referred to as the father of this science, and his little book, "Formulary for the use and preparation of many new drugs," published in 1821, is considered a classic.

Among other facts he showed that strychnine when injected, although it may be carried in the blood stream throughout the body, has its chief effect on the nervous system. The question of how it was absorbed, and whether it was absorbed solely through the lymphatics, as the famous Hunter brothers in England would have claimed, led him to experiment on the digestive tract, particularly on the mechanism of swallowing and vomiting, work which is still often mentioned in textbooks of physiology. His description of the symptoms shown by animals to which he had administered food containing no nitrogen has led at least one author to suggest that Magendie be given credit for foreshadowing the possibility of deficiency diseases, *i.e.*, avitaminosis. His name is also associated with an investigation as to the origin of cerebrospinal fluid, and the opening he discovered between the

subarachnoid space and the ventricle of the brain is still called Magendie's foramen.

For the present day physiologist, however, Magendie's name is remembered for his work on the direction of conduction in spinal nerve roots. He showed that impulses are conveyed towards the spinal cord over the dorsal roots, which are therefore sensory, and away from it over the ventral roots, which are therefore motor. This fundamental finding provided a pattern for mapping out the functions of the nervous system, a part of the body exploration of which has proved somewhat baffling to investigators not only of that era but even of the present time. Although Charles Bell, after Magendie announced his findings in 1821, claimed that he had priority to this discovery, and consequently it is now generally known as the Bell-Magendie Rule, I have elsewhere given my reason for believing that the credit should go to Magendie, in spite of one author's taking me to task for my stand, since he said there was glory enough in this discovery for both Bell and Magendie.

One other finding of Magendie's is worthy of mention. In his day it was considered that if sugar was found in a patient's blood he was suffering from diabetes. With the new methods of analysis available toward the middle of the century he found that glucose was a normal constituent of mammalian blood, a fact which he rightly said would be of great importance for the study of diabetes.

By 1820, therefore, Magendie had successfully launched physiology along the experimental way and the return to the 17th century attitude exemplified by Harvey, Boyle and others, instead of the more speculative attitude of von Haller and Boerhaave of the 18th century, led to the enormous advances of the nineteen-hundreds.

Magendie had followers but also rivals as well. Chief of the rivals was Fluorens who was rash enough to undertake investigations on the nervous system, an area to which, as we have seen, Magendie laid claim. Fluorens at almost the very time that Magendie was working on the spinal nerve roots began his experiments on the cerebral hemispheres and cerebellum of the pigeon, and his description of the behavior of birds after removal of these parts of their nervous system is classic. It must be re-

marked that Magendie also worked on the cerebellum and is given credit for the concept that this organ is concerned in maintaining the equilibrium of the body both in the erect posture and in walking. This was because of the forced movements he observed when lesions were made in this part of the nervous system, the details of which were worked out later by others. Fluorens did not confine his experiments to birds but later extended his work to mammals. However, birds were his favorite subjects and while investigating deafness produced in the pigeon upon destroying different parts of its auditory apparatus, he found that interference with the semicircular canals did not produce symptoms of deafness, but changes in the bird's movements, not only in its ability to maintain the equilibrium of its body in standing and flying, but also in eye movements as well. We now designate as Fluorens' Law his finding that "stimulation of a semicircular canal elicits nystagmus in the plane of that canal." He took up the problem of the location of the respiratory center where Legallois had left it, and succeeded in narrowing down this area, so necessary to life, to a very small spot in the medulla. Upon puncturing this area in the frog, he found that instead of the invariably fatal effect of this operation on warm-blooded animals, the frog can survive because of sufficient exchange of gases through its moist skin. One other of Fluorens' investigations should be mentioned. He was the first to cross-suture nerves successfully, and as one would imagine he chose the bird for his experimental animal.

During the 1830's Magendie and Fluorens were the center of activity in French physiology, but we find mention in the comprehensive textbooks, which were the fashion in the early part of the present century and which seem to have tried to rival one another in the number of pages they could contain, of certain other worthwhile observations made in France at this time. For example, Luciani claims that Bequerel and Brechet in 1835 were the first to make observations on the heat produced in human muscles during their contraction, by using a newly invented thermo-electric battery attached to the biceps. Such observations show the activity of the period but a list of the names and the usually single items attached to them have their place

in those more comprehensive treatises to which I have referred rather than in the brief account I am able to present here.

Let us turn to Magendie's celebrated pupil Claude Bernard, who in his own life-time was referred to by a celebrated politician as being not a physiologist but physiology itself. Indeed, at the present time I am sure that when French physiology is mentioned, Claude Bernard's name comes at once to mind. He did not begin to publish until 1843 when he was thirty years of age, but once launched on his career over 300 publications appeared under his name, including 15 volumes of lectures delivered at the Collège de Frane and the Museum of Natural History, as well as his famous *Introduction to the Study of Experimental Medicine*. He began his career with an investigation of the origin of the small nerve running across the inner surface of the tympanic membrane, the chorda tympani, then went on to discoveries regarding pancreatic digestion, the glycogenic function of the liver, vasomotor nerves, the action of such poisons as curare and carbon monoxide, the fact that plants as well as animals are susceptible to anaesthetics, and a host of other findings which his incessant experimentation brought to light. He coined the expression "internal secretion" to characterize the situation he had found in the liver, since he noted that this organ not only forms bile, a secretion which flows out through a duct to the exterior, but also glucose which leaves the liver by way of the blood, and thus initiated the science of endocrinology. One of his ideas has been likened in importance to Darwin's theory of evolution, namely his Doctrine of the Internal Environment, a concept to which Walter Cannon applied the term homeostasis, a word which now appears in every textbook of physiology.

Bernard continued working up to the very time of his death early in 1878, and after his death his faithful followers found among his papers some notes of experiments on alcoholic fermentation. These they precipitately published, much to the distress of Pasteur, for Bernard held the opinion that fermentation was in the last analysis chemical action, while Pasteur was convinced that fermentation could only take place through the agency of *living* yeast cells.

Bernard's successor to the chair of medicine at the Collège de

France was a most picturesque character, Charles-Edouard Brown-Séquard, born on the Isle de France, now called Mauritius, son of an American ship captain and a French lady. He had his medical training in Paris, but was at one time professor at the Medical College of Virginia in Richmond, and at another, professor at Harvard, before he settled down at the Collège de France in Paris. His earliest and one of his most important discoveries was first touched upon in the thesis he wrote for his M.D. degree in 1846, but several years of experimentation were necessary to complete the full story; this was his finding that sensory impulses from touch, pain, heat and cold after they have reached the spinal cord via the dorsal nerve roots, pass to the other side of the cord, then up to the brain by paths other than the dorsal columns, as Charles Bell thought he had established. We now apply the term "the Brown-Séquard Syndrome" to the condition where the spinal cord has been hemisected and sensation is lost on the opposite side of the body, below the level of the lesion, and movement is lost on the same side. Brown-Séquard is also noted for having in 1852 anticipated Claude Bernard by three months in showing that stimulation of the sympathetic nerve to the rabbit's ear produced vasoconstriction. Bernard had already shown that cutting this nerve led to flushing of the ear, and when he reported that applying an electric current to this nerve caused the ear to become pale, he was much surprised to be told that Brown-Séquard had already published an account of this phenomenon in an American medical journal. Bernard did not read English! Brown-Séquard also played an important part in the discovery of the importance to life of certain of our endocrine organs, for in his hands, animals deprived of their adrenals died within a matter of days. We now find that with *proper treatment* such animals can be kept alive. At the end of his life he became most enthusiastic over hormonal treatment, and believed that the extract he obtained from the testes of animals was a panacea for a host of human diseases.

Bernard, who dominated French physiology from 1840 to 1870, was distinctly more interested in the chemical aspects of our science; witness his ten years' struggle to work out the complete story of the glycogenic functions of the liver, *i.e.*, the form in which the carbohydrate substance was stored and the

conditions under which it was changed to glucose and released to the blood stream. For it had taken him that long a time before he was finally able to isolate the hypothetical substance to which he had given the name glycogen, *i.e.* sugar former. Although one of his favorite pupils, d'Arsonval, was especially skillful with electrical apparatus, Bernard himself seems not to have been very excited over measuring blood pressures or engaging in other problems which would demand the use of physical rather than chemical means of approach. One of the most successful French physiologists of this period to use physical methods was Antoine Chauveau, and some authors would place him in the front rank with Bernard, although his name may not be too well known to most of us. He was younger than Bernard, having been born in 1828, and lived on into the 20th century. He was for 30 years professor in the Veterinary School at Lyons, and was appointed inspector general of veterinary schools in France. With Marey, Chauveau in the 1850's constructed marvelous instruments for showing up the action of the heart and blood vessels, and because he had horses at his disposal as experimental animals, the results were most striking. Chauveau did not confine his attention to the circulatory system, but experimented on the cranial nerves, locating their origin in the brain, and showing the part played by the 10th cranial nerve in swallowing. One author flatly states that to Chauveau and not to Bernard should go the credit for the discovery that the liver releases sugar, but since the whole idea of the glycogenic function of the liver originated with Bernard, and no one in the history of physiology has worked out so complete a story of a bodily function as he did in this case, I do not believe priority on this one point detracts from our high opinion of Claude Bernard. Chauveau became one of the earliest and most ardent defenders of Pasteur, and his work on attenuation of bacterial action, immunization, and especially on tuberculosis, would seem to me to place Chauveau more in the field of bacteriology than physiology.

The names of Broca and Vulpian should not be omitted in this account—Broca because he showed the site of the lesion in the cerebral hemispheres which causes motor aphasia; Vulpian for continuing the work of Bernard on vasomotor nerves, and particularly for his published lectures which did a great service in

assembling the scattered observations of Bernard, and his associates, as well as his own investigations on the physiology of the nervous system.

The person who is always characterized as Bernard's favorite pupil, and who, when Bernard approached the age of 60, took over his chair at the Sorbonne—Bernard always occupied simultaneously chairs in two or three different Paris institutions, the University, the Collège de France, the Museum of Natural History—this pupil is also known as the "Father of Aviation Medicine," Paul Bert. Bert worked for two years as Bernard's research assistant in his laboratory at the Collège de France before starting out on his own. To him we owe our knowledge of the conditions necessary for the successful grafting and transplantation of tissues, but it was in the field of respiration that he did his most striking work. He had chambers built for compression and decompression of human subjects, and discovered the fundamental principle that ill effects of high altitude are not due to diminished mechanical pressure but to the diminished partial pressure of oxygen. In the 1870's balloon ascensions were arousing great interest in France, and Paul Bert's contributions to the effects of altitude on the human body were most timely. However, he became so involved in politics after the Franco-Prussian War that he abandoned his physiological investigation and, after serving as Minister of Education in France, accepted the post of resident general in French Indo-China. His colonial policy there is considered more humane and helpful than had ever before been followed, and it is only today, 70 years after his death from dysentery in Hanoi, a few months after his arrival there, that we read in our newspapers of a revolt of Indo-China from the status of a French colony.

This brief account has necessarily omitted many names and the individual discoveries linked with them. I have endeavored, however, to show how it was that experimental physiology got its start in the 19th century through the influence of François Magendie, and that the impetus he gave it was transmitted on through his pupils and his pupils' pupils.

If a statistician were to count the number of times the expression "was the first to show that" appears in a modern textbook of physiology the size of the well-known Best and Taylor,

I would wager that more French names would precede this phrase than those of any other nationality, provided the discovery referred to had occurred in the half century between the years 1810 and 1860, and that the name of Claude Bernard would top the list.

# CELLULAR IMMUNITY AS DEMONSTRATED IN TISSUE CULTURES WITH VIRUSES*

MORRIS POLLARD

Animals respond to antigenic stimuli with antibodies which can be demonstrated in the serum by special techniques. Such antibodies reflect the functional capacity of cells which are associated with the reticulo-endothelial system (RES). This field of interest has been reviewed extensively by Chase (1953) and by McMaster (1953). Extracts of lymphoid tissue and homografts of lymphoid tissue have engendered serum antibody levels in recipient animals, and of these the intact cells were superior (Fagraeus and Grabar, 1953; Harris, Harris and Farber, 1954). Antibodies thus conferred have been demonstrable by *in vitro* techniques and by challenge tests *in vivo* (Stavitsky, 1952; Harris and Harris, 1954; Ginder, 1955).

Antibody production in tissue culture, as described by Carrell and Ingebrigtsen (1912), has been confirmed and denied by several subsequent reports. A factor emphasized in affirmative reports was the need for living cells, since extracts of the same tissues failed to yield significant levels of antibody (Parker, 1938; Fagraeus, 1948).

The effects of viral agents on tumor explants range through a spectrum from the tissue-destruction by St. Louis encephalitis (SLE) and Western equine encephalomyelitis viruses, to the stimulating effect of rabies and yellow fever viruses (Pollard and Bussell, 1952; Pollard, Bussell, and Snyder, 1954). It was observed that SLE and mouse encephalomyocarditis (EMC) viruses exerted marked destructive effects on mouse tumors, regardless of the immunological status of the tissue donor. When SLE and EMC viruses were inoculated into spleen tissue explants cytopathogenic effects could be observed in all explants except those derived from animals which had been immunized against the same virus. Thus an immunological function of

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spleen tissue appeared to be reflected in its capacity to ward off a destructive sequence following exposure to virus.

## *Methods*

Twenty CFW mice were immunized against SLE virus by two weekly intraperitoneal inoculations of 1000 $LD_{50}$ of virus, followed by an intracerebral challenge with 100 $LD_{50}$ of the same virus. Twenty CFW mice were immunized against EMC virus by 4 intraperitoneal inoculations of formalin-killed virus, then one subcutaneous challenge inoculation with 10 $LD_{50}$ of the same virus plus a final challenge of 1000 $LD_{50}$ subcutaneously. Seventy per cent of the mice thus immunized against EMC succumbed to the first challenge; however, the survivors resisted subsequent challenge of 1000 $LD_{50}$ EMC virus intracerebrally.

Spleens from normal mice and from the immunized mice were individually processed, as follows: each spleen was minced with scissors so that all pieces of tissue were approximately 1 square mm. in size. These were washed three times in Hanks solution and finally suspended in chick embryo extract-Hanks solution (1+3 proportion). Approximately ¼ of each spleen was explanted in chicken plasma onto the wall of a 15 mm. roller tube. One ml. of nutrient fluid (serum UF,[1] Hanks solution, embryo extract) was added to each tube. The nutrient fluid contained penicillin (1200 units per ml.) and streptomycin (10 mg. per ml.). The tubes were sealed with rubber stoppers and rotated at 6 revolutions per hour at 36° C. Two days after the tissues were explanted the nutrient fluid was decanted and replaced. Two tubes each of spleen were then inoculated either with 100 $LD_{50}$ of ECM virus or of SLE virus. Baseline virus determinations were performed by intracerebral inoculation of the infective nutrient fluid into mice and the fate of the virus was similarly determined after 3, 5, and 7 days.

The spleen explants were observed and photographed with low power magnification through the wall of the tube and the changes in the explants were recorded daily.

[1] Microbiological Associates, Bethesda, Maryland.

*Results*

On the first and second days after spleen explantation there was considerable peripheral migration of blood cells in each explant. After the nutrient fluid was exchanged, observation on most of the explants revealed lymphoid cells either surrounding them or grouped at one pole of the tissue particle.

In explants of normal spleen tissues infected with SLE and EMC viruses the round cells at the periphery of each explant became pyknotic and fragmented. The infected explants appeared dead after the third day but an occasional explant infected with SLE virus appeared to resume growth 5 to 6 days later. EMC virus persisted in the nutrient fluids but SLE virus could not be detected after the fifth day.

Spleen explants from EMC-immune mice were unaltered by EMC virus, but they were fragmented and lysed by SLE virus. Conversely spleen explants from SLE-immune mice were unchanged by SLE virus, but they were destroyed by EMC virus. Virus was not detectable in the nutrient fluid of the unaltered explants on the third day (Figs. I and II).

*Discussion and Summary*. Viral agents have been propagated in adenoid tissues, but it is significant that optimal multiplication of virus occurred in tissues from which fibroblasts had been

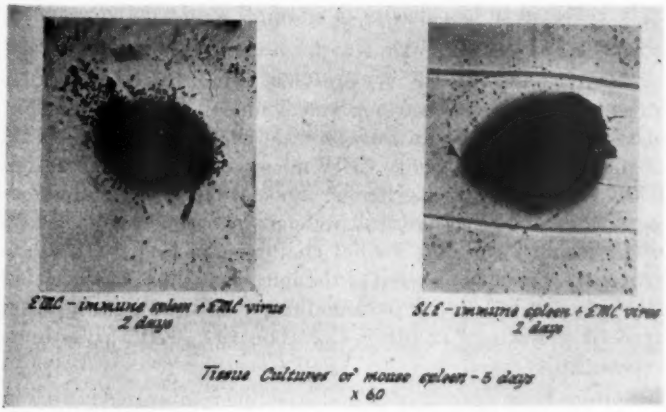


FIGURE I

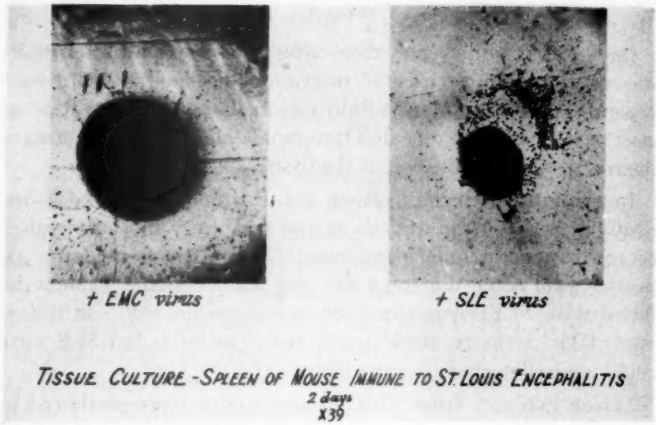


FIGURE II

nurtured (Southam and Babcock, 1954). When poliomyelitis virus was inoculated into tissues at the time of explantation, little or no multiplication of virus occurred (Barski *et al.*, 1953). After SLE, Western equine encephalomyelitis and cytopathogenic effects could be observed in all explants except those derived from animals which had been immunized against the same virus. Thus an immunological function of spleen tissue appeared to be reflected in its capacity to ward off a destructive sequence following exposure to virus.

*Methods.* Twenty CFW mice were immunized against SLE virus by two weekly intraperitoneal inoculations of 1000 $LD_{50}$ of virus, followed by an intracerebral challenge with 100 $LD_{50}$ of the same virus. Twenty CFW mice were immunized against EMC virus by 4 intraperitoneal inoculations of formalin-killed virus, then one subcutaneous challenge inoculation with 10 $LD_{50}$ of the same virus plus a final challenge of 1000 $LD_{50}$ subcutaneously. Seventy per cent of the mice thus immunized against EMC succumbed to the first challenge; however, the survivors resisted subsequent challenges of 1000 $LD_{50}$ EMC virus intracerebrally.

Spleens from normal mice and from the immunized mice were individually processed, as follows: each spleen was minced with

scissors so that all pieces of tissue were approximately 1 square mm. in size. These were washed three times in Hanks solution and finally suspended in chick embryo extract-Hanks solution (1+3 proportion). Approximately ¼ of each spleen was explanted in chicken plasma on to the wall of a 15 mm. roller tube. One ml. of nutrient fluid (serum UF[1], Hanks solution, embryo extract) was added to each tube. The nutrient fluid contained penicillin (1200 units per ml.) and streptomycin (10 mg. per ml.). The tubes were sealed with rubber stoppers and rotated at 6 revolutions per hour at 36° C. Two days after the tissues were explanted the nutrient fluid was decanted and replaced. Two tubes each of spleen were then inoculated either with 100 $LD_{50}$ of EMC virus or of SLE virus. Baseline virus determinations were performed by intracerebral rabies virus were inoculated into fresh mouse spleen suspended tissue cultures, they could not be recovered later than the third day following inoculation of virus (Pollard, 1954).

As described above spleen tissue explants have revealed a specific cellular resistance to virus challenge, which was a reflection of the immune status of the tissue donor. This cellular reaction to virus invasion may be interpreted as a manifestation of the immune response as observed with the intact challenged animal. The reaction of spleen tissue explants to virus challenge may provide a sensitive technique for antigenic analysis but obviously it is demonstrable only with cytopathogenic agents. By selecting two such agents for challenge experiments on spleen explants, immunity was demonstrable by failure of a virus to exert a cytopathogenic effect and by coincident rapid disappearance of the virus from the nutrient fluid.

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# THE MOLLUSCAN EYESTALK: USING AS AN EXAMPLE, PTEROCERA LAMBIS

J. H. PRINCE*

*Pterocera lambis* is known as the 'Spider shell' in the area in which the specimens used in this investigation were caught. The maximum size encountered at the site appeared to be about sixteen inches long including the spines, usually seven in number and approximately equal to the body length. The specimens used were approximately six inches long, and they came from the coral reef of Green Island, a pure atoll of the Great Barrier Reef, seventeen miles off the east coast of Northern Queensland, Australia. They were taken in winter from water 80° Fahrenheit.

The scientific name is a little obscure. 'Pterocera' may stem from the Greek word 'Pteron' meaning a 'wing' or 'fin', which may apply equally well to the shape of either the shell or the foot; and the Greek word 'Keraia' meaning a 'horn' or 'projection', which undoubtedly refers to the spines. The second word may be related to the Latin word 'Lambo' meaning to 'lick' or to 'bathe', and may refer to the animal's habit of lying in shallow rock pools.

This mollusc, which has relatives in many parts of the world (a similar species *Pterocera scorpio L.* is found around the Philippine Islands), has two eyes mounted on stalks or pedicles known as 'omnatophores', which can be retracted right back into the shell and body, and which when extended can be controlled sufficiently to permit a form of convergence onto an object. Sometimes this visual fixation enables the animal to strike accurately with its razor-like foot. But it must be conceded that the foot is used mainly for locomotion, and that its use as weapon is quite secondary. It is plunged into the coral or sand, and muscular contraction then pulls the shell and body after it.

In addition to the eye, each omnatophore carries an olfactory tentacle or horn, and a small sensory node. There are three

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FIG. I. Pterocera lambis, 2/3 actual size.

muscular systems arranged round a central sinus which extends from a reticulo-endothelial plexus at the base of the stalk to a cavity surrounding the eye at the tip, and which sends smaller branches in all directions. There are mottled markings on the eyestalk similar to those on the body. Sometimes these are concentric bands of varying colour, but golden-yellow, brown, and silver usually predominate.

The extension of the omnatophore and the eye at the end of it is partly by fluid engorgement; haemolymph or blood entering a central sinus or chamber through the stalk and around the eye. The exact mechanism of this engorgement is not wholly clear, but undoubtedly the main factors are dilation of the fluid sinus

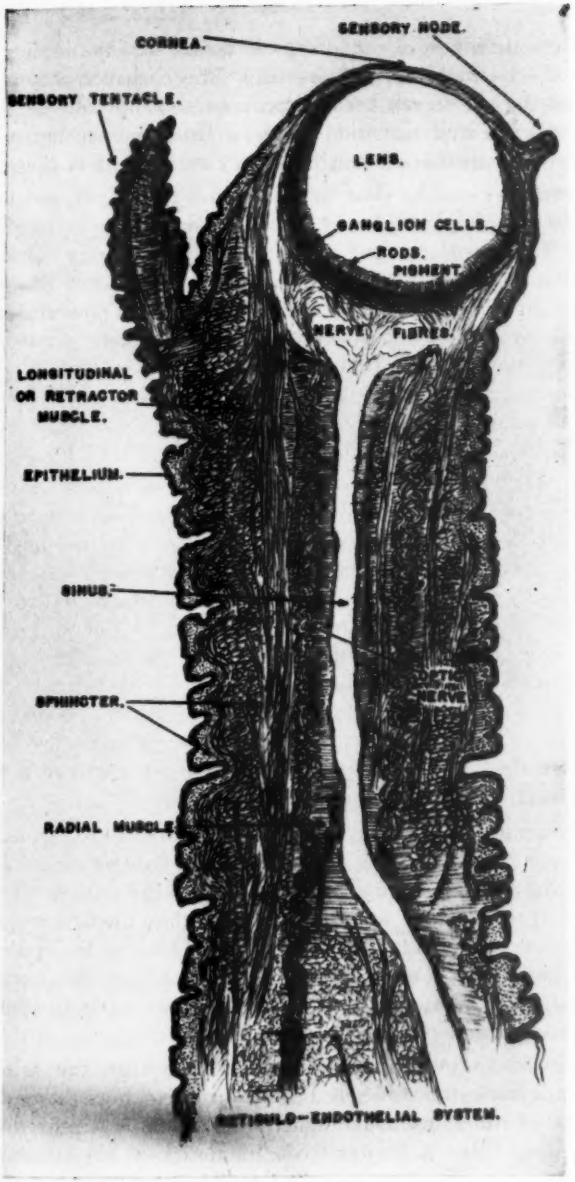


FIG. II. The omnatophore, showing the sensory tentacle, sensory node, sinus, reticulo-endothelial system, and muscular systems.

by the contraction of radial muscle bands, and the forcing into it of the haemolymph by pressure. The chamber around and behind the eye serves a second purpose, to supply all the necessary oxygen and nutrition to the retina, the sensitivity and specialised function of which are very demanding of these.

Retraction can be slow and voluntary, or rapid and in the nature of a reflex response to touch, odour, or the cutting off of light. But extension of the stalk appears to be entirely voluntary, and the result too of a general body relaxation. A relaxation of the longitudinal (retractor) muscles permits the powerful radial muscle bands to dilate the sinus. At the same time, these radial bands gather the longitudinal muscles into a tight mass, adding to the rigidity of the stalk. There is no obvious indication that any of these actions are under hormone control. Relaxation of the organ comes after death.

MUSCULATURE. There are three main muscular structures around the central sinus in the eyestalk. They consist only of unstriated fibers of varying thickness. This is in keeping with general molluscan musculature. The main directions of the muscles are *Sphincter*. This encircles the stalk in two main layers, with numerous smaller groupings of fibers. The first sphincter layer is sub-dermal, and this is the smaller and finer of the two main ones. The second layer is separated from the first by a layer of longitudinal muscle, and this second layer consists of more definite bundles and coarser and more powerful structure. The other sphincter groups are scattered between longitudinal and radial muscle bands.

*Retractor*. The first layer of longitudinal muscle has already been mentioned as being located between the two main sphincters, and the second one lies internally to the second sphincter layer. It too is larger and more powerful than the first longitudinal layer which undulates more with the folds of the epidermis. This large muscle sends out branches in oblique directions into the adjacent sphincter layers. Then there are a few smaller longitudinal muscles interlacing the radial muscles.

It is this muscle which withdraws the eye into the stalk, and the stalk back into the shell. But it must have more than a single source of innervation, for apart from the lateral movement it furnishes, there is frequently a traction in a prescribed area,

bending the stalk into a curve, the better to view a close object, and even to permit a form of convergence of the two stalks.

*Radial.* This is located mainly around the central sinus, and radiates outwards to find a firm anchorage on, and in the longitudinal muscles. A few sphincter bundles bind it where it is

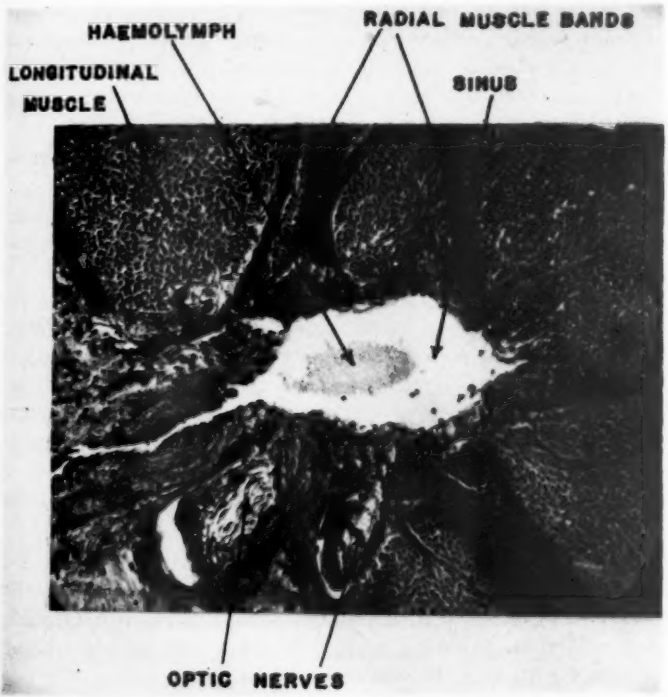


FIG. III. Radial muscle bands around the central sinus. x 100.

adjacent to the central sinus. It seems certain that the radial and sphincter muscles contract when the longitudinal or retractor muscles relax, and in this way they enlarge the sinus diameter whilst restricting the diameter of the whole eyestalk, and increasing its rigidity. This permits an influx of haemolymph to the sinus and its branches, which in turn helps to extend the eyestalk and add even further to its rigidity.

Sinus. The eye at the end of the omnatophore is almost wholly surrounded by a cavity or post-ocular sinus which narrows to closure behind the corneal margin, and extends posteriorly as the large central sinus down the entire length of the eyestalk. This sinus is branched, and serves many lymph spaces throughout the stalk. It has a dual function.

1. It contributes towards extension and swelling of the stalk by filling with haemolymph from a large plexus at the base of the stalk. It also provides an avenue for nutrient material and oxygen to reach the whole of the eye, the loose connections of which afford it ready access.

2. It permits great contraction of the stalk, and retraction of the eye within the stalk as it empties of haemolymph.

The optic nerve fibers bridge the post-ocular sinus in numerous bundles which form separate optic tracts to the cerebral ganglion.

Vascular Plexus. This is a rather inappropriate term for what is undoubtedly a haemolymph reservoir and filter of reticulo-endothelial material. It is a large network at the base of the eyestalk which is very obviously lymphatic material or its homologue. It consists of connective tissue with large cavities containing haemolymph. There is a typical mosaic pattern of staining showing endothelium, and there are large numbers of giant macrophages of a fixed type which usually line cavities and sinuses within the connective tissue.

Perhaps it would be more accurate to call these cells, not macrophages, but Reticulo-endothelial system ('R.E.S.') cells. In any case, much of their function is to ingest zoospores, algae, and all other irritating organisms from the animal's bloodstream, for these can be seen within them.

The Eyes. Among invertebrates, and especially those with only simple eyes, the eyes of *Pterocera lambis* may be surpassed in performance only by those of the cephalopods which seldom have less than three times as many receptors per unit area. They are certainly comparable in performance with those of a large number of vertebrates. Apart from the fact that the retina is unreversed, these eyes are deficient in only one important feature possessed by vertebrates, accommodation. And even this may be achieved in an indirect manner.

*Retina.* This membrane consists of four layers, with a thickness of 0.264 mm. in the center, and 0.115 mm. at the periphery. The four layers are:

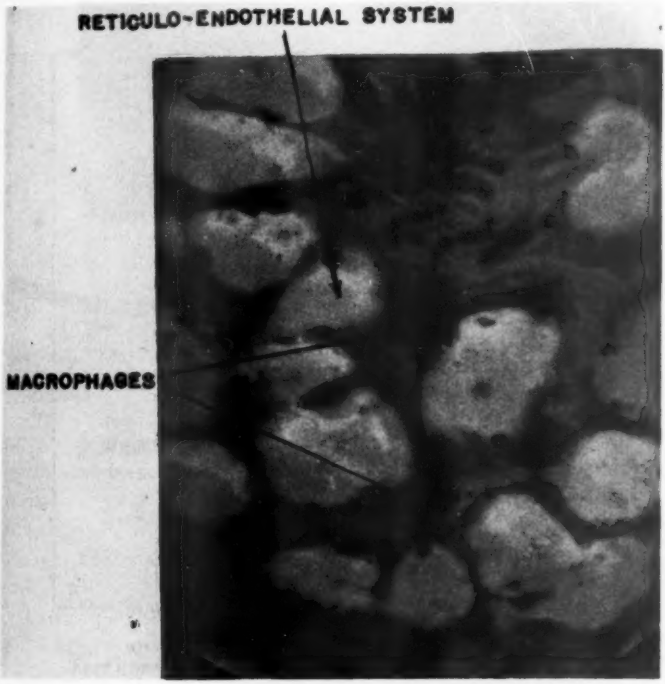


FIG. IV. The reticulo-endothelial system showing large R.E.S. cells. x 250.

1. *Receptors.* These are best described as rods so far as their shape is concerned, and they are reasonably uniform in size, and of only one kind. The outer segments are slender, with a central filament or extended cytoplasmic structure tapering to a point near the tip within the rod, and having slightly denser staining properties than the rest of the segment. This is reminiscent of the nerve fiber being continuous through the receptor with a cilium, as in the eyes of some of the lower invertebrates. To some extent this may be refractile.

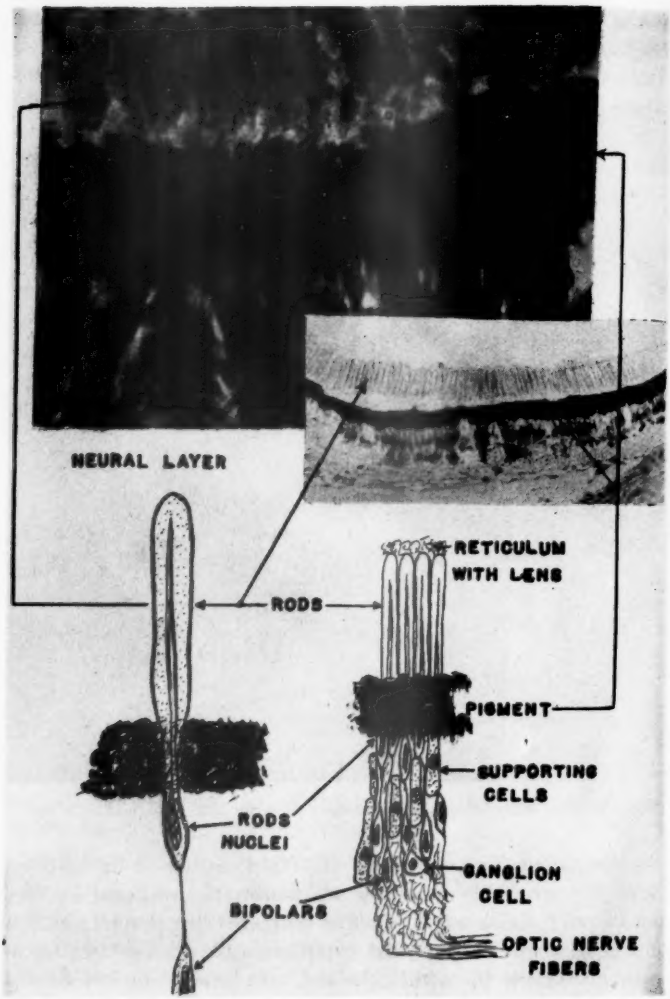


FIG. V. The retina, showing mainly the pigment, the rods, and the position of the layer of supporting and neutral cells. x 1000; inset x 100.

This filament or core is quite strong, for when a rod is broken, it often remains projecting from the base of the rod. In odd instances, the filament spreads at the tip. There is no obvious function for this core to each rod, but it is possible to make one further suggestion; it could be in some way homologous with the rhabdom structure found in the ommatidia of compound invertebrate eyes.

The habits of the animal, and the bulk of the outer segments of the rods suggest that they have a fairly low threshold. They are a little shorter and thicker at the periphery of the retina as are those of the cephalopods, and here they are probably more sensitive to movement and light intensity than to image quality.

The rod nuclei are partially within and beyond the pigment which constitutes the second layer of the retina, and these nuclei resemble the bipolar cells found near them, being very elongated. The sizes of the receptors range from 0.0825 mm. in length at the center, to 0.033 mm. at the periphery, and the average thickness is 0.011 mm.

This suggests a population of something approaching 10,000 receptors to the square millimeter, and in view of the curvature of the cornea which is in contact with the pupil, and the fact that the eye is mounted on a moveable pedicle, it would enjoy a reasonable field of view, although not necessarily a very sharp image. The quality of image is probably comparable to those of some of the sharks and rays, but an equivalent number of receptors per unit area could be more efficient in a larger eye, so the comparison is not an accurate one anyway. And in any case it compares very weakly with the 200,000 receptors to the square millimeter found in the human fovea, which makes its quality even more difficult to visualise.

2. *Pigment.* The retinal pigment is local, forming a shield between the rods and the rest of the retina. The pigment cell nuclei are buried within the pigment which has no detectable migratory power. Its obvious task is to prevent stray light from being intercepted by the rods when coming from directions other than the pupil, and also to prevent light from passing straight through beyond the eye.

The pigment consists of dense particles of melanin distributed

thickly around the rods, and in some cases it encroaches round the rod nuclei. The pigment layer is 0.033 mm. thick.

3. *Layer of neural and supporting cells.* In this layer, which is also thicker in the center of the eye (0.0825 mm.), it is possible to distinguish bipolar cells, a few horizontal cells, most of the rod nuclei, and ganglion cells in a proportion of one to five bipolar cells. But the bulk of all the cells in the layer appear to be homologous in function, although somewhat different in form with Muller's fibers in the vertebrate retina.

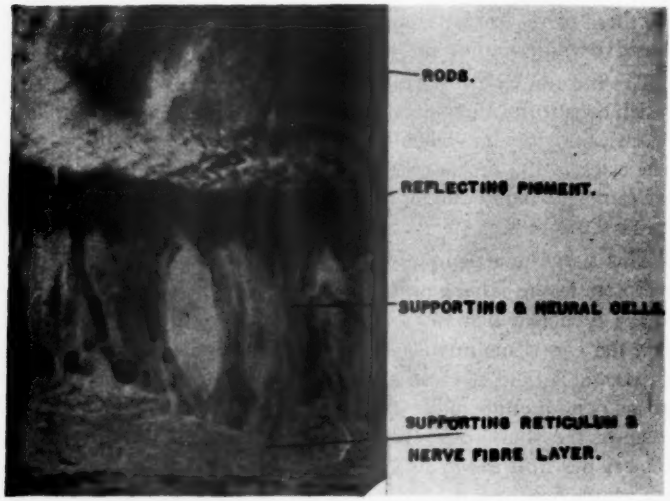


FIG. VI. The retina, showing the layer of supporting and neural cells. x 500.

The bipolar cells are slender and elongated, and their nuclei appear to be virtual replicas of the rod nuclei. They send their axons on to the optic tract, except where they synapse with the ganglion cells. The horizontal cells are fewer in number, and may have some kind of summating function, though this must be primitive in form compared with that of the vertebrates. The supporting cells, which form two not very distinct rows, are long, and to some extent resemble columnar epithelium. Their processes appear to form the supporting network for the next layer. A similar appearance is encountered in the pseudo-stratified epi-

thelium of the early embryonic stages of the human brain and spinal chord, which retain neural cells within their structure.

The increased central thickness of this layer coupled with the greater length of the rods in the same area suggests a form of central vision which is superior to that at the periphery. Some of the nuclei, usually the bipolars, cluster together in groups and send their individual axons right through to the optic nerve.

4. *Layer of optic nerve fibers.* This layer, which is 0.066 mm. thick, consists of the axons of the bipolar and ganglion cells in layer 3. The fibers gather together in small bundles to pass through the supporting network of this layer, and then these join into larger bundles which, after turning in various directions, eventually form the external limiting membrane and leave the eye in numerous optic nerves or fascicles.

Optic Nerves. The optic nerves, which have thickness varying up to 0.165 mm., are folded and extensible whilst they traverse the eyestalk on their way to the cerebral ganglion. The fibers in these nerve tracts vary in thickness, and they are accompanied by numerous cells possessing elongated nuclei, and which are, or are homologous with, oligodendroglia. They are definitely neuroglial.

When the nerves pass through the reticulo-endothelial system, they are protected by thick jackets of large macrophage-like cells which are obviously endowed with the task of protecting the nerves from irritating and damaging organisms, for these cells are found to be well-filled with ingested material.

Cornea. The epithelium of the omnatophore becomes adapted as corneal epithelium by thinning and assuming a more regular formation. The cells become shorter, more rectangular, and devoid of accompanying chromatophores, the proportion of length to width becoming a two to one ratio. The nuclei also become less elongated. The lamina propria is thinner in the center of the cornea, and is bounded by a fine limiting membrane which stains darkly with haematoxylin and eosin, and which is probably homologous with Bowman's membrane of the vertebrate cornea.

The stroma or lamina propria is in two layers, distinguishable only by the fact that the posterior part stains more deeply than the anterior, and may therefore have a higher refractive index. There is no definite endothelial layer lining the cornea, but there

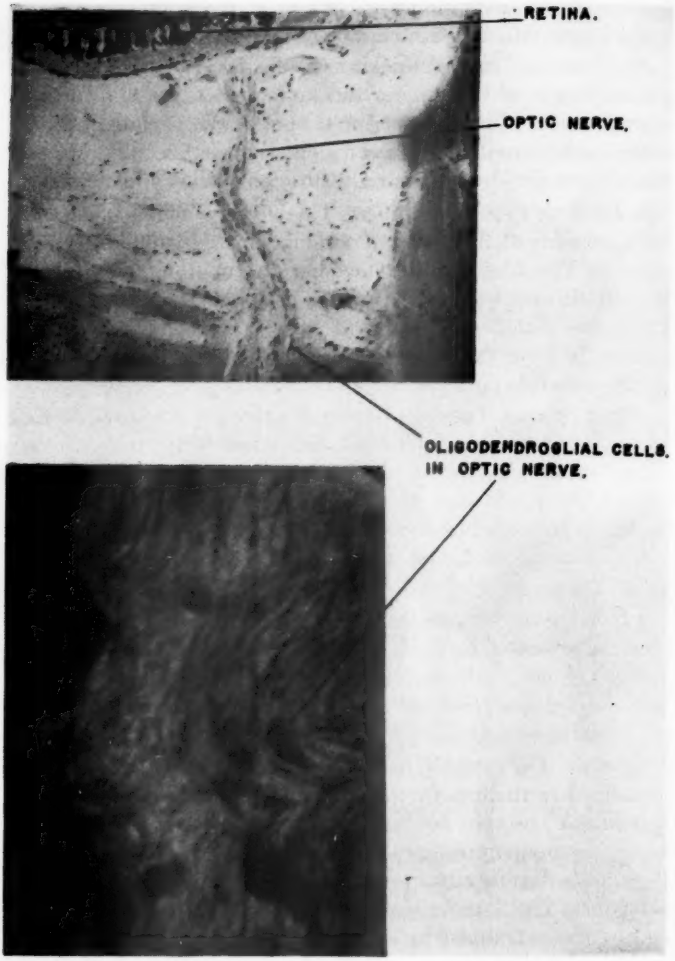


FIG. VII. The optic nerves, showing how they bridge the post-ocular sinus, and the oligodendroglia within them. x 90; x 900.

is a second slight and very thin lightening of the stroma, and the cell walls are a little stouter and more clearly defined on the rear surface. The stroma is continuous with the subdermal muscular layer, especially the darker staining portion.

PUPIL. There is no true iris, and the pupil is formed by a post-corneal aperture in the retinal pigment, no provision for glare control normally being required because of the animal's environment, and the protection afforded by the shell.

LENS. The lens, which has a diameter of 1.023 mm., occupies the whole cavity of the eye, and although after fixation, it has what may be called a 'formation' it is devoid of cellular structure. It is a vitreous-like material with a greater density in its center, and a condensed layer of capsular vitreous material on its outer surface. It is best described as a hyaline metachromatic body.

It is joined to the tips of the rods by a fine reticulum, and the attachment this makes is so powerful that the rods can be pulled from the rest of the retina with the lens. The network does not appear to extend to the posterior surface of the cornea, although undoubtedly the lens is ordinarily in contact with it.

The condensed layer of vitreous material which is the homologue of a vertebrate lens capsule, is rather wider than would be expected of a capsular membrane, and evidently has a higher refractive index than the material within it. Although the lens has no cellular formation, it breaks up into a regular pattern when fixed to a point of hardness. This is what was meant by 'formation.'

There is no accommodatory apparatus, and the most advantageous visual distance appears to be from the center of the animal's body to the fullest extension of the foot. But some degree of focussing adjustment may be obtained with the extension of the omnatophore. If this stalk is pressed forward to its limit, the pressure from the fluid in the post-ocular cavity would tend to compress the eye slightly from behind, thus shortening its axis, and giving a better conjugate focus for objects at a greater distance. Relaxation of this tension would permit a slight lengthening of the globe of the eye with a consequent better adjustment for short distances. As the animal tends to relax the omnatophores when inspecting close objects, in fact sometimes even bending them slightly, this hypothesis is logical.

OLFACTOR. Each omnatophore (12 mm. long in the specimens under examination) has a small horn or pointed protuberance (3 mm. long) situated medially and slightly behind the eye, and this extends as far forward as the eye. It is generally agreed that this organ is olfactory in the Helicidae, in *Limax*, and in *Arion*,

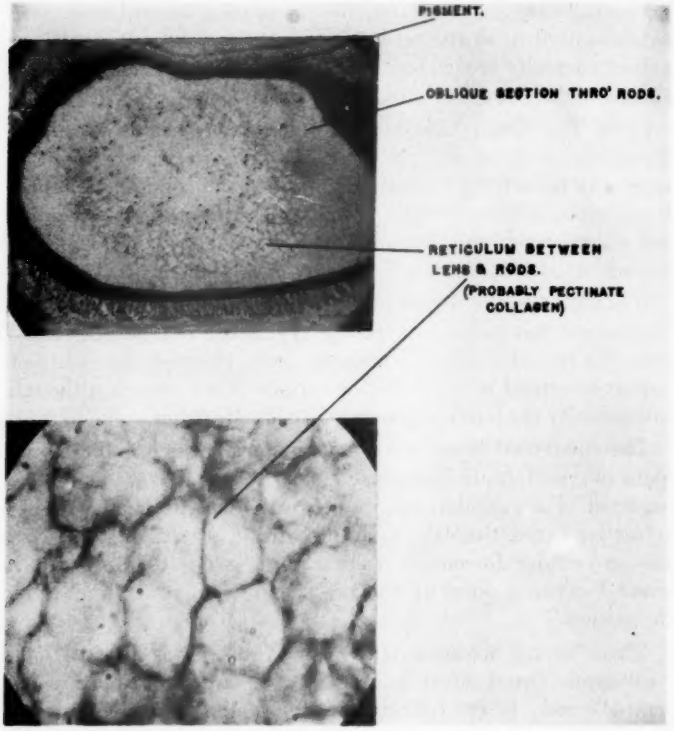


FIG. VIII. The network, probably pectinate collagen, joining the lens with the rods. x 120; x 1000.

and its neural structure and connections in *Pterocera lambis* suggests a similar function. Certainly its habits suggest that it has a keen sense of smell.

The olfactory horn extends and retracts on exactly the same mechanical principles as the eyestalk, but there is evidence that it is not retracted to the extent that it cannot be used as a cover or shield for the eye when this is withdrawn. In well-developed specimens it is very reminiscent of the extra-brillar fringes of some geckos.

There are numerous large club-shaped cells which are either tactile or olfactory, and the ends of which protrude from the

surface epithelium of this olfactor. Most of these are found within the folds of the epithelium. They are reminiscent of the sense cells of insect hairs, but are more likely to be chemoreceptive. Each one is something like a *sensillum basonicum*. The nucleus is at the base of the cell, away from the exterior of the epithelium, and the cells appear to develop from prominent round ones at the basal membrane of the epithelium.

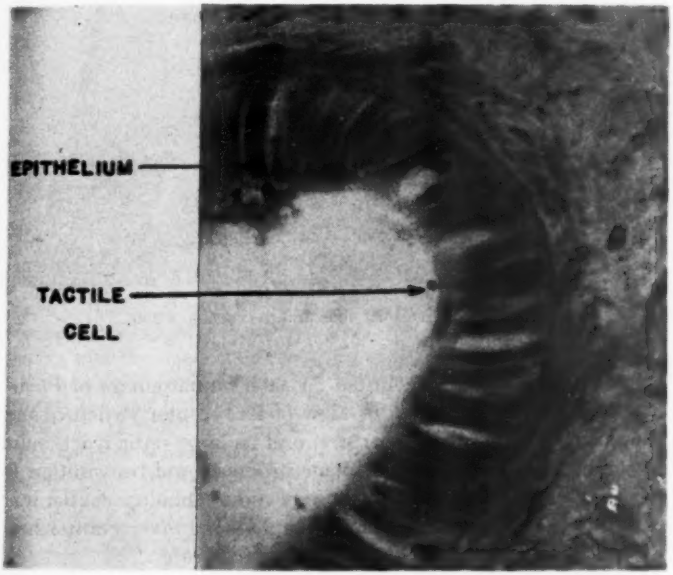


FIG. IX. The epithelium of the olfactor, showing tactile cell. x 350.

SENSORY NODE. Nearer to the eye, but situated laterally, is a small sensory node, 0.25 mm. long and 0.2 mm. wide which does not appear to possess any contractile power. It may have some value in interpreting the environment by a sensitivity to the chemistry of the surrounding water whilst the eye is withdrawn and vision impossible, but on the other hand, histological examination, and its location in direct continuation with the cornea of the eye, suggest that its main function is merely tactile. It is certainly in the most suitable position for that.

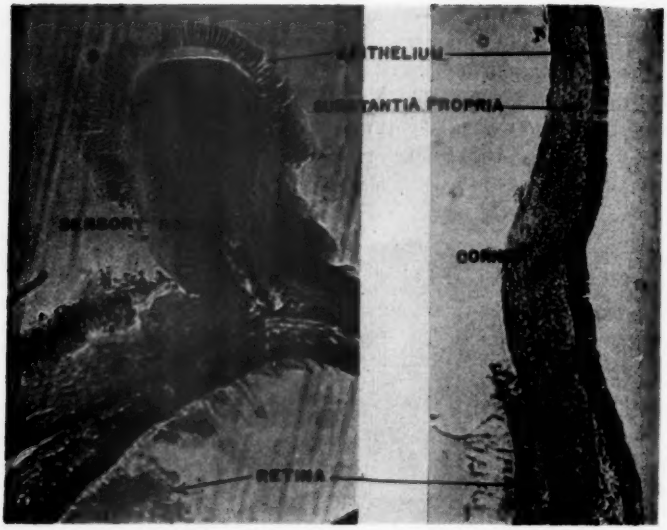


FIG. X. The sensory node and the cornea. x 250.

SUMMARY. There is mounted on each ommatophore of *Pterocera lambis* an eye which, in view of its receptor structure and population, its pupillary aperture, and its large optic tract, must be reasonably efficient as an image producer and transmitter. It may not be comparable with those of most cephalopods, but it is certainly one of the most efficient found among invertebrates, and is even comparable with those of many vertebrates.

The eye is of the simple invertebrate form with an unreversed retina, one kind of receptor, a partially summated system, and multiple optic nerves. The lens is structureless, a vitreous body joined to the receptors by a connective network. There is no accommodatory apparatus, and the pupil is an aperture in the retinal and epithelial pigment. The cornea is a transparent window in the surface epithelium. They are described histologically.

At the edge of the cornea, on the lateral side of the eye, there is a sensory node which is evidently tactile in function. Medially and slightly behind the eye there is an olfactory horn which may also act as a protective shield for the eye when it is withdrawn.

The eyestalk is motivated by three sets of muscles, retractors, radial, and sphincter, and these are all situated round a central haemolymph sinus which widens to form a cup-like cavity behind the eye, and which at the base of the stalk connects with a reticulo-endothelial system having the task of filtering the haemolymph, and acting as a reservoir for it. The sinus fills with haemolymph to aid in extending the omnatophore, and to nourish the eye and the other tissues.

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## ABSTRACT

*Pterocera lambis* is a mollusc which possesses two eyes mounted on omnatophores. Adjacent to the eyes are also olfactory and tactile nodes. The stalk possesses three sets of muscles, retractors, sphincters, and radials. The retractors withdraw the eye and stalk, the sphincters endow it with rigidity, and the radials dilate the central haemolymph sinus, which then fills with haemolymph and forces the eye out to its fullest extent, and adds to the rigidity of the stalk. This sinus enlarges post-ocularly and is the route by which the retina is nourished.

The eye is of the unreversed kind, without an active iris, and with no accommodatory apparatus, any accommodation possible being only by variation in position of the eye, and adjustment of its length by pressure from the post-ocular sinus. The receptors are of one kind only, rods. They appear to have a reasonably low threshold on the basis of bulk and the habits of the animal; their distribution being of the order of 10,000 to the square millimeter.

The retina has little summation, the rod nuclei being connected to bipolars, and to ganglion cells in the ratio of about one ganglion cell to five bipolars. There is a strong supporting structure to the retina, consisting of epithelial cells homologous with Muller's fibers in the vertebrate retina. The optic nerve fibers gather into several bundles, and after crossing the post-ocular sinus, pass down the omnatophore to the appropriate ganglion. They are plentifully supplied with oligodendroglia. The lens of the eye is of the vitreous kind, and without cellular structure.

At the base of the eyestalk there is a reticula-endothelial system which filters the haemolymph of zoospores and other irritants. The R.E.S. cells are thickly distributed around the optic nerves too.

# PROCEEDINGS, TEXAS BRANCH OF THE SOCIETY OF AMERICAN BACTERIOLOGISTS

DECEMBER 3–4, 1954

THE UNIVERSITY OF TEXAS, AUSTIN

Studies on Indirect U.V. Irradiation in Relation to Mutagenic Activity. Carl Wiesen and Orville Wyss, The University of Texas, Austin.

A Study of Metabolic Reactivation of Bacteria Inactivated by Ultraviolet. M. D. Socolofsky and Orville Wyss, The University of Texas, Austin.

Induced Pigmentation in *Serratia marcescens*. R. P. Willioms and J. A. Green, Baylor Medical School, Houston.

A Comparative Study of the Porphyrin Constituents of Chlorophyll Mutants of Green Algae. L. H. Krough, Jr., L. L. Smith, and R. B. Mefferd, Jr., Southwest Foundation for Research and Education and Trinity University, San Antonio.

Bacteriological Studies Related to Certain Phases of Cheese Ripening. I. I. Peters and A. V. Moore, Texas A. & M., College Station.

The Effect of Soil Sterilization, Inoculation, and Phosphorus on Symbiotic Nitrogen Fixation in Houston Black Clay Soil. R. J. Hervey, Texas Agriculture Experiment Station, Temple.

Studies on Thiamine Chloride Hypersensitivity. W. J. Fahlberg and C. D. Dukes, Baylor Medical School, Houston.

Mutual Influence of Sensitizing and Challenge Tumors Upon One Another as Influenced by the Dosage Size. J. D. Flintjer, P. Mazel, and R. B. Mefferd, Jr., Southwest Foundation for Research and Education and Trinity University, San Antonio.

The Arthus Reaction Actively and Passively Induced in the Mouse. A. A. Benedict and R. L. Tips, The University of Texas Medical Branch, Galveston.

Studies on the Mechanism of Germination at 75° C. of Mesophilic *Clostridium* Spores. E. S. Wynne and L. I. Galyen, M. D. Anderson Hospital, Houston.

The Effect of Administering Antibiotics Upon Antibiotic Resistant Microorganisms in the Intestinal Tract of the Chick. J. F. Elam, R. L. Jacobs, W. L. Tidwell, and J. R. Couch, Texas A. & M., College Station.

Inhibition of Chlortetracycline Activity in the Intestinal Tract of the Rat. G. E. Peterson, University of Houston, Houston.

Potentiation of Experimental Monilial and Staphylococcal Infections by Tetracycline. Irene S. Tettenbaum, V. A. Hospital, and C. D. Dukes, Baylor Medical School, Houston.

Studies on the Mechanism of the Fungistatic Action of Salicylates. J. B. Nash, L. Gutierrez-Vela, and G. A. Emerson, The University of Texas Medical Branch, Galveston.

Enzymatic Formation of Meso-Tartaric Acid From the Mold Product Trans-L-Succinic Epoxide. W. R. Martin and J. W. Foster, The University of Texas, Austin.

Studies on the Isolation and Physiology of Some Bacteria Utilizing Gaseous Hydrocarbons. M. Dworkin and J. W. Foster, The University of Texas, Austin.

Studies on Viral Hepatitis in Embryonated Eggs. M. Pollard and L. T. Diserens, The University of Texas Medical Branch, Galveston.

An Investigation of Encephalitis. J. V. Irons, Minnie Lee Sung, and Thelma D. Sullivan, Texas State Department of Health, Austin.

Additional Studies on Psittacosis. Thelma D. Sullivan, J. Grimes, K. Knolle, and D. Mason, Texas State Department of Health, Austin.

*Pseudomonas* Infections in Burn Patients. I. An Attempt to Classify Recently Isolated Strains. J. A. Rivera and Ruth Latta, Brooke Army Medical Center, Fort Sam Houston.

Observation on a New Species of *Haemophilus*. C. D. Dukes and H. L. Gardner, Baylor Medical School, Houston.

Effects of Added Cysteine on Growth of *Streptococcus Faecalis* in Over-Autoclaved Medium. Cora L. Gott and E. S. Wynne, M. D. Anderson Hospital, Houston.

Total Phosphorus as an Indication of Bacterial Mass. E. O. Bennett, University of Houston, and R. P. Williams, Baylor Medical School, Houston.

A Blue Pigment Isolated from *Serratia marcescens*. J. A. Green, R. P. Williams, and D. A. Rappoport, Baylor Medical School, Houston.

Finding of *Balantidium coli* in Human Stool. H. E. Henderson, Texas State Department of Health, Austin.

Nitrogen Fixation and Hydrogenase in Bacteria. H. Wynn and Orville Wyss, The University of Texas, Austin.

Auto-Immune Disease in Humans. S. Haberman, J. M. Hill, and D. E. Soules, Wadley Research Institute and Blood Center, Dallas.

Some Dynamics of Agglutination and the Commbs Antiglobulin Reaction. S. Haberman and D. E. Soules, Wadley Research Institute and Blood Center, Dallas.

The Role of Starch in a Synthetic Medium for *Hemorphilus pertussis*. P. Donaldson, Southwestern Medical School, Dallas.

Clinical Use of the Gradient Plate Method for Antibiotic Sensitivity Testing. A. I. Braude, J. Banister, and N. Wr'ghts, McKinney V. A. Hospital, McKinney.

## I. M. LEWIS LECTURE

Dr. Clarence F. Schmidt
Continental Can Company, Chicago, Illinois

"Problems in the Study of Sporulation by Members of the Genus *Bacillus*"

## ABSTRACTS

Indirect Action of Ultraviolet Irradiation. Carl Wiesen and Orville Wyss, The University of Texas, Austin.

Bacterial culture media subjected to non-ionizing radiations vary in their mutagenic action on bacterial cultures depending on the accumulation of organic peroxide in such broths. While a large number of substances can contribute to this accumulation the lipid substances are particularly effective. Different broths show quantitative specificity in inducing mutations in the several loci studied indicating that the mutagenic action is due to the organic peroxide and not to the hydrogen peroxide in equilibrium, $RO_2 \leftarrow \rightarrow H_2O_2$. Comparisons of various broths reveal that some contain antimutagenic substances.

A Study of Metabolic Reactivation. Marion Socolofsky and Orville Wyss, The University of Texas, Austin.

Recently, the phenomenon of metabolic reactivation has been described in bacteriological literature. This phenomenon is based upon the observation that a bacterial culture, which has been irradiated by ultraviolet light, increases in viable cell count when incubated for 24 hours in the presence of various members of the citric acid cycle. This observation was confirmed in that cultures killed down to $10^4$ viable cells per ml. yielded $10^6$ cells after the metabolic reactivation treatment. Well controlled experiments indicated that a major factor involved in the increase in viable cell count may be the multiplication of the cells which survived the irradiation. An unirradiated culture of *Escherichia coli*, diluted to $10^4$ cells per ml. and placed in a solution of C.P. phosphate and citric acid cycle intermediates for 24 hours, will grow to $10^6$ cells in that time although no nitrogen source is supplied. Computation of the amount of nitrogen required for such growth indicates that such amounts may be present in the solutions of highly purified chemicals. Confirmation of this view was obtained from rate studies and from experiments involving an adenineless strain of *E. coli*. In the latter case the "reactivation" occurred only when adenine was added to reactivating treatment.

Induced Pigmentation in *Serratia marcescens*. R. P. Williams and J. A. Green, Baylor University College of Medicine, Houston.

The red strain, Nima, of *Serratia marcescens* is extremely stable. However, ultraviolet light or gamma radiation sources will induce both white and orange mutants from the parent Nima strain. We have isolated an ultraviolet light induced white mutant and a gamma radiation induced orange mutant. These mutants are both stable producing only orange and white colonies. By accident an unusual relationship was discovered between the orange and white mutants. The orange mutant excretes a diffusible substance that causes the white mutant to turn red. The syntrophic pigmentation is directly related to the proximity of the two organisms; the closer they are, the quicker the appearance of color in the white colony. The amount of pigmentation in the white colony is directly related to the proximity of the organisms and the incubation time; the closer they are and the longer the incubation time, the greater the amount of pigmentation. These results indicate the orange mutant is blocked in its ability to produce red pigment, but accumulates a diffusible precursor which the white mutant can utilize to complete its formation of pigment. A study of this relationship should lead to a better understanding of pigment production in *S. marcescens*.

A Comparative Study of the Porphyrin Constituents of Chlorophyll Mutants of Green Algae. L. H. Krough, Jr., L. L. Smith, and Roy B. Mefferd, Jr., Southwest Foundation for Research and Education, and Trinity University, San Antonio.

Chlorophyll mutants of two species of green algae were obtained by isolation following ultraviolet irradiation. Two mutants of *Chlamydomonas pseudagloe* (olive green and orange) and two of *Chlorella pyrenoidosa* (light green and yellow) were incubated in light at 24° C. on Casitone-agar medium. The cultures were harvested at two weeks, washed, homogenized, and extracted in the dark with cold methanol.

Extinction coefficients (methanol) indicated that total absorptive material was markedly reduced in all mutants and that there was little or no chlorophyll a or b in any mutant. Some 12 to 13 visible or fluorescing spots separated in paper chromatography

with 5 per cent n-propanol in ligroin from the two parent strain solutions, while only one was evident in the Chlorella mutants and four in the Chlamydomonas. There was no free chlorophyll b detectable in any of the four mutants, and a very small quantity of chlorophyll a in each of the Chlamydomonas mutants. There were distinct alterations in the carotenes and xanthophylls in all the mutants as well as in the porphyrins. It appears likely that the biochemical block has affected early processes in each case, which indirectly influence a number of biosynthetic sequences, including the porphyrins, carotenes, and xanthophylls.

The Effect of Soil Sterilization, Inoculation and Phosphorus on Symbiotic Nitrogen Fixation in Houston Black Clay. Ralph J. Hervey, Texas Agriculture Experiment Station, Temple.

Many workers have reported the beneficial effects of inoculation and of phosphorus on both root-nodule bacteria and legume plants. Recent radio-phosphorus studies with sweet clover in the highly calcareous, black soils of Texas confirm the beneficial effect of P on legume growth. To answer the question: Is P primarily influencing *Rhizobia* or legume, microbiological studies, to determine the effect of various methods of inoculation and the role of phosphorus in symbiotic nitrogen fixation, were initiated at the Blackland Station.

Experiments to date indicate that inoculation and method of inoculating (whether to seed or soil) has, generally, much less influence on nodulation, plant growth and quantity of plant nitrogen than phosphorus. Nodulation of sweet clover increased many fold in phosphated soil and nodules were larger. The P effect was especially marked in autoclaved soil where heating of the highly calcarious soil apparently made residual P almost completely unavailable. Yields of plant tissue and nitrogen markedly increased in phosphated soil, although percentage nitrogen was changed little. Sterilized soil without P allowed little growth.

Phosphorus increased markedly the free *Rhizobia* in sterilized soil, and to some extent *Rhizobia* and the total microbial population (bacteria and actinomncetes) in unsterilized soil. Microbial antagonism appears to be a factor inhibiting *Rhizobia* in natural soil.

Mutual Influence of Sensitizing and Challenging Tumors Upon One Another, as Influenced by the Dosage Size. J. D. Flintjer, P. Mazel, and R. B. Mefferd. Southwest Foundation for Research and Education and Trinity University, San Antonio.

Experiments were performed to determine the influence of different sizes of sensitizing and challenging viable tumor grafts upon each other in homologous hosts. On day one, half of 160 closely-bred, Sherman-strain, white rats were sensitized with 40 mg. and the other half with 6.5 mg. fibrosarcoma fragments.

On day eleven, these two groups were each divided into three subgroups. Paired subgroups, each pair consisting of an equal number of animals sensitized with large and small tumors, respectively, were then challenged with three sizes of tumor fragments: 6.5, 29, and 40 mg. fragments. At the same time 20 controls were inoculated with 6.5 mg. and 15 controls were inoculated with 40 mg. fragments to determine tumor growth in the absence of another tumor. Differences in takes and weight increases of resultant tumors were measured to determine results.

Since the variance of the ratios of tumor gain is exceedingly large in each of the experimental groups, while the variance in the controls is very small, it appears that the ability to develop resistance patterns actually is a continuous spectrum in other than highly inbred strains. It also appears that homologous tumor implants and subsequent implants of the same tumor in the same host are influenced by immunologic responses of the host.

Studies on the Mechanism of Germination at 75° C. of Spores of Mesophilic *Clostridium* Species. E. Staten Wynne and L. I. Galyen, The University of Texas M. D. Anderson Hospital and Tumor Institute, Houston.

At the last meeting of this society, evidence was presented that spores of mesophilic *Clostridium* species underwent rapid germination at 75° C. in glucose which had been autoclaved for 80 minutes in the presence of phosphate. Subsequent work has shown that glucose can be replaced by fructose, mannose, arabinose, xylose, lactose, or maltose, but not by sucrose. However, cysteine and thioglycollate were inactive. Phosphate could be replaced by $Na_2CO_3$, $K_2CO_3$, or $CaCO_3$. In fact, germination occurred in the absence of any buffer, provided the glucose was

sufficiently alkaline to insure that the pH was kept alkaline or slightly acid during autoclaving.

Germination was not observed in lactate, pyruvate, malate, or oxalate. Although hydroxymethylfurfural supported germination of all 5 species tested, it is considered unlikely that germination in autoclaved carbohydrates can be explained by formation of this compound. Production of furans from sugars has been reported to proceed chiefly at pH values of 3 or lower, and germination did not occur when autoclaving was carried out under such conditions.

Inhibition of Chlortetracycline Activity in the Intestinal Tract of the Rat. G. E. Peterson, Department of Biology, University of Houston.

A study was made to determine why aureomycin fails to inhibit bacteria in the intestinal tract of the rat to the extent that it does *in vitro*. The agar plate-disk method which employs *Bacillus cereus* No. 5 for the assay of aureomycin revealed that feces of rats fed 100 ppm. dietary aureomycin contain many times the amount of acid-acetone and/or water extractable antibiotic required to inhibit completely the growth of isolated intestinal bacteria *in vitro*. However, water extracts of feces contained only about 10 per cent as much aureomycin as did acid-acetone extracts. Furthermore, experiments *in vitro* showed that only 24 per cent of the aureomycin which had been added to a sterile 10 per cent aqueous suspension of normal feces could be recovered from the water phase after 72 hours incubation, whereas, 76 per cent was bound in the fecal sediment and had to be extracted with acid-acetone. Perhaps the antibiotic bound by the intestinal contents may be unavailable for antibacterial action, thus, greatly reducing the apparent effective concentration of the drug in the intestinal tract.

When the aureomycin sensitivities of several lactobacillus, enterococcus and coliform isolates from the feces rats receiving dietary aureomycin were compared with those of similar isolates from control animals, it was found that the antibiotic regimen caused a moderate increase in the aureomycin resistance of these bacteria. However, *in vitro*, even the most resistant isolates could tolerate only a fraction of the water extractable drug in the feces from which they were derived.

The spread plate method of detecting antibiotic reversal revealed a rather high "reversal factor" for aureomycin in normal rat feces, thus, suggesting that another factor responsible for the inhibition of aureomycin *in vitro* is the presence of certain growth factors in the intestinal tract.

*Pseudomonas* Infections in Burn Patients. I. An Attempt to Classify Recently Isolated Strains. Jose A. Rivera and Ruth Latta, Brooke Army Medical Center, Fort Sam Houston.

This report covers the incidence of *Pseudomonas* infections on a burn ward. *Preudomonas* infections in burn patients are persistent and do not tend to decrease in frequency or virulence despite local and systemic antibiotic treatment. This may be due to the widespread use of some antibiotics which inhibit growth of other organisms, particularly in mixed infections. There have been a few fatal cases of septicemia due to *Pseudomonas*. Many strains of Polymixin B-resistant *Pseudomonas* have been isolated at this Unit.

The classification of 350 recently isolated *Pseudomonas* is being delayed by discrepancies and lack of consistency in the current literature in regard to reports on cultural characteristics and important biochemical reactions. This report includes new media and tests for nitrate reduction and pigment production. The authors conclude with a recommendation for a more consistent method as the basis for sound criteria in classifying *Pseudomonas*.

Effects of Added Cysteine on Growth of *Streptococcus faecalis* in Over-autoclaved Medium. Cora L. Gott and E. Staten Wynne, The University of Texas M. D. Anderson Hospital and Tumor Institute, Houston.

Suboptimal growth of *Streptococcus faecalis* R occurred when the medium had been sterilized by filtration, or by autoclaving at 121° C. for 5 minutes or 40 minutes. With over-autoclaved medium (40 minutes at 121° C.), addition of cysteine *before* autoclaving resulted in growth equal to that in unsupplemented medium which had been autoclaved for an optimal period (10 minutes). Cysteine added to over-autoclaved medium *after* autoclaving was without effect. In optimally autoclaved medium, cysteine was stimulatory if added before autoclaving, but with-

out effect if added afterwards. Cystine yielded results equivalent to those obtained with cysteine. Supplementation of over-autoclaved medium with comparable quantities of all amino acids in the medium other than cysteine resulted in partial restoration of growth, regardless of whether the addition was before or after autoclaving.

Total Phosphorus as an Indication of Bacterial Mass. E. O. Bennett, University of Houston, and R. P. Williams, Baylor University College of Medicine.

Several methods are available for determining bacterial mass, among them being turbidity, viable or total counts, and nitrogen determinations. Chain formation in *Bacillus anthracis* and the presence of nitrogen in the capsule preclude the use of the above methods for accurate determination of bacterial mass in *B. anthracis*. Since phosphorus is a universal protoplasmic constituent we decided to use it as a measure of bacterial mass. Preliminary experiments showed that of the various phosphorus fractions present in bacterial cells, total phosphorus gives the best measure of bacterial mass. To evaluate our measure we have correlated total phosphorus determinations with turbidity, viable count, and Kjeldahl nitrogen determinations in *Escherichia coli*, *Micrococcus pyogenes*, *Bacillus subtilis*, and *Preudomonas aeruginosa*. In every case total phosphorus measurements showed a positive correlation with the accepted methods of turbidity, viable count, and Kjeldahl nitrogen. Our results indicate that total phosphorus is a reliable method for determining bacterial mass when used under standardized conditions.

A Blue Pigment Isolated from *Serratia marcescens*. R. P. Williams, J. A. Green, and D. A. Rappoport, Baylor University College of Medicine, Houston.

The pigment of *Serratia marcescens*, prodigiosin, has been described as a red substance with a greenish metallic luster. Previous workers have regarded prodigiosin as a simple substance. We have evidence that the pigment is not a single substance. Strain Nima of *S. marcescens* was grown on the surface of a glycerol-inorganic salts medium in several Roux bottles. Cells were harvested and pigment extracted by acetic acid-acetone treatment. Extracted pigment was chromatographed by circular paper chromatography using petroleum ether, ether, and heptane as solvents. The chromatograms indicated several pigment

fractions existed in prodigiosin. There is a blue fraction, at least two red fractions, and an orange-red fraction. The red and orange-red fractions can be separated as a single band from the blue by use of proper solvent mixtures. Ultraviolet and visible spectra of the red-orange-red mixture and the blue fraction are patently different. Infra-red spectra of the two pigment fractions show they are similar but have important differences. The chromatographic, ultraviolet and visible spectra, and infra-red spectra indicate that at least two distinct fractions exist in prodigiosin extracted from the Nima strain of S. marcescens.

Hydrogenase and Nitrogen Fixation in Freshly Isolated Strains of Nitrogen-fixing Organisms. J. H. Wynn and Margaret Green, University of Alabama, University, Alabama.

One hundred and nineteen strains of azotobacter were isolated from Alabama soils. In contrast to previous reports in the literature, many of these cultures produced considerable acid during growth in sucrose nitrogen-free broth even when aeration was supplied by a rotary shaker. However, this low pH did not inhibit the activity of nitrogenase; $N_2$ was fixed in cultures after the pH mm. was 5.0.

Seventy-nine of the above cultures were tested for hydrogenase, the enzyme which had been postulated as a coupled system supplying reduced intermediates for the system fixing nitrogen. Only 27 per cent of these cultures had detectable hydrogenase. Cultures which were positive for hydrogenase had a final pH above 6.6; cultures which were negative were more acid. When certain cultures which had no hydrogenase were tested at an earlier stage of growth before the pH fell to 6.6, hydrogenase was detected. These observations have led to the suggestion that hydrogenase is not essential to the fixation of nitrogen by growing cultures. They do not eliminate the possibility that the systems can be coupled; but it is possible to inhibit or destroy hydrogenase in cultures in which nitrogenase is still active.

Five cultures, tentatively identified as *Arthrobacter* spp., grew in one hundred serial transfers in nitrogen-free media and were, therefore, assumed to be nitrogen-fixing bacteria heretofore unknown. The absence of a detectable hydrogenase in these cultures further supports the conclusion that nitrogenase may function in the absence of hydrogenase.

# STUDY BY ELECTRON MICROSCOPY OF ERYTHROCYTES FROM A PATIENT AFFECTED WITH SARCOIDOSIS*

REGINALD L. REAGAN, EDDY D. PALMER,† EDWARD C. DELAHA, AND A. L. BRUECKNER

Sarcoidosis has been variously described as a type of tuberculosis, as a disease caused by acid-fast bacilli not yet identified, as a disease entity characterized by proliferation of reticulo-endothelial cells, as a systemic tumor of multicentral origin, and as the product of an unknown virus. The best supported opinion, however, is that the etiology remains unknown.

The individual lesion of sarcoidosis is a tubercle-like structure, approximately the size of a miliary tubercle, and consists almost exclusively of large epitheloid cells which are frequently fused. Lymphocytes are, as a rule, found only in the periphery, and probably belong to the normal organ structure in which sarcoid involvement has occurred and not to the sarcoid tubercle itself. In contrast to tuberculosis, caseation does not belong to the pathognomonic picture of sarcoidosis, although minute central foci of caseation are found not infrequently, provided many different foci are examined in many sections. Healing occurs spontaneously in a good many cases, either by resorption or fibrosis. Again in contrast to tuberculosis, fibrosis rarely leads to encapsulation; rather, the entire granulomatous lesion is transformed into a fibrosed nonspecific nodule, consisting of broad hyalinized fibers without nuclei or nuclear debris. Various inclusion bodies have been described, but they are not pathognomonic of sarcoidosis. They may be manifestations of the causative agent. The sites of predilection are lymph nodes, lung, skin, bone marrow, liver, spleen, kidneys, and myocardium, approximately in the order named. Relatively rare sites are the mammary glands, adrenals, and other glands of internal secretion.

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† Chief, Gastrointestinal Section, Walter Reed Army Hospital.

CASE REPORT: A 19-year-old Negro soldier was hospitalized because of occasional aching pain in the right chest upon exertion. This was not at all incapacitating, and there had been no systemic complaints. Physical examination was normal except for mild bilateral supraclavicular lymphadenopathy. Tuberculin, histoplasmin, and coccidioiden skin tests were negative. The chest film showed wide-spread densities with soft margins through both lung fields, and the roentgen impression was sarcoidosis. Bronchoscopy, bronchial washings and gastric washings were normal. Liver biopsy and gastric mucosal biopsy showed scattered minute granulomas, but specific identification could not be made. Several small nodes were removed from the neck, and these showed the typical histopathologic picture of sarcoidosis. Venous blood was drawn for virologic study. The patient was observed for five months. He remained afebrile and appeared to be well. His pain did not

FIG. I. Electron micrograph of an erythrocyte showing virus-like bodies, designated by arrows, from a patient affected with Sarcoidosis. Shadowed with chromium at arc tangent 2/12. x 25,000.

return. Serial chest films showed no change. The tuberculin test remained negative.

*Materials and Methods.* Five cc. of venous blood was collected from the patient and placed in sterile 50 cc. Erlenmeyer flasks which contained 7 mg. of heparin in 6 cc. of sterile physiological saline. Ten cc. of venous blood was drawn likewise from a patient with no history of having had this disease (control). The flasks of infected heparinized blood and the flask of normal heparinized blood were then placed in the —4° C. ice box for one hour. They were then removed from the ice box and allowed to stand at room temperature for ten minutes with occasional rotation of each

FIG. II. Electron micrograph of an erythrocyte showing virus-like bodies, designated by arrows, from a patient affected with Sarcoidosis. Shadowed with chromium at arc tangent 2/12. x 20,000.

flask. Several drops of each suspension were placed on several parlodion film supports which had been prepared 48 hours previously. After all excess fluid had been removed with small capillary pipettes, the films were then washed with sterile distilled water, dried and shadowed with chromium (Williams and Wyckoff, 1946) at arc tangent 2/12 and examined under the R.C.A. electron microscope, Type E.M.U.

### *Results*

Upon examination of the specimen under the electron microscope, no virus-like particles were observed in the erythrocytes from the normal control group in approximately 2,000 fields examined. Figures I, II, III, and IV are electron micrographs show-

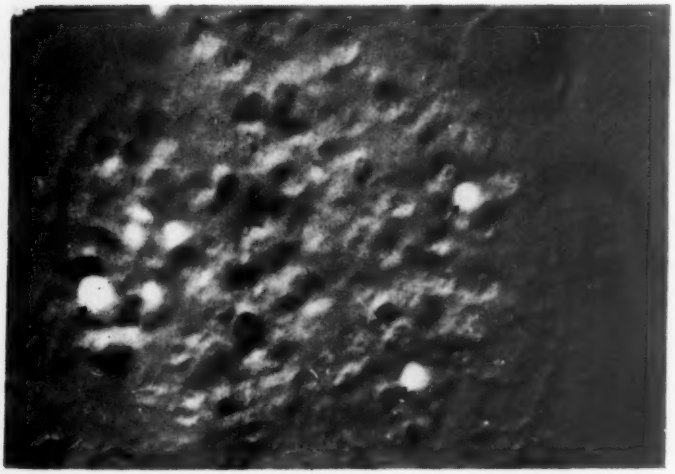

FIG. III. Electron micograph of an erythrocyte showing virus-like bodies, designated by arrows, from a patient affected with Sarcoidosis. Shadowed with chromium at art tangent 2/12. x 20,000.

ing virus-like particles on the surface of and in the erythrocytes of the sarcoidosis patient. The majority of these particles are spherical in shape and measure 240 to 250 mμ by direct measurement. Irregular contours may be seen on some particles. Figure

V shows an erythrocyte from the normal blood containing no virus-like particles.

FIG. IV. Electron micrograph of part of an erythrocyte showing virus-like bodies, designated by arrows, from a patient affected with Sarcoidosis. Shadowed with chromium at arc tangent 2/12. x 40,000.

*Summary.* Studies by electron micrographs of erythrocytes from a patient affected with sarcoidosis disease show the virus-like particles to be spherical with irregular contour, and to be on or somewhat in the erythrocyte. These virus-like particles have a diameter of 240 to 250 mμ. The bodies could not be demonstrated in normal human blood subjected to the same procedure of preparation and electron microscopic examination. These bodies re-

semble the virus of measles and the virus bodies of infectious mononucleosis described by Reagan *et al.* (1952, 1953).

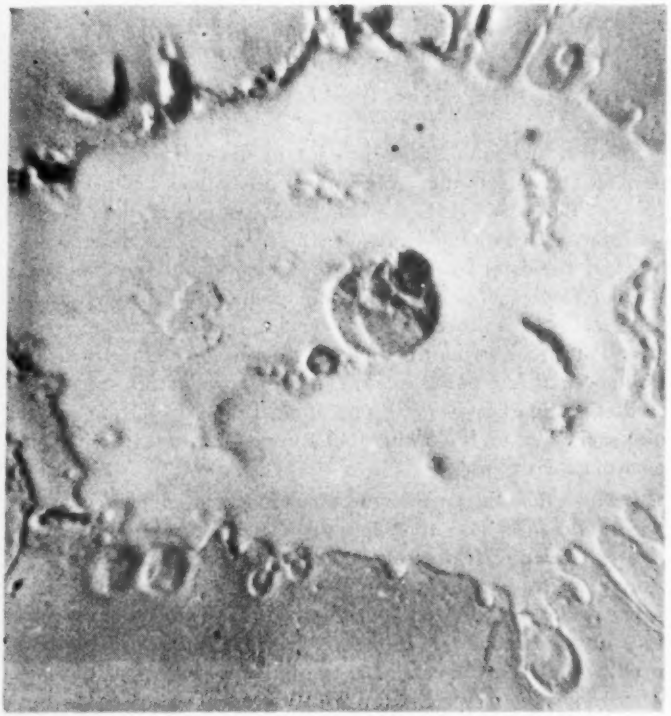

FIG. V. Electron micograph of an erythrocyte from a normal patient, shadowed with chromium at tangent 2/12. x 40,000.

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# STUDIES BY ELECTRON MICROSCOPY OF A FIXED (PASTEUR) RABIES VIRUS, A STREET RABIES VIRUS AND THE EGG ADAPTED FLURY STRAIN OF RABIES*

REGINALD L. REAGAN, SING CHANG, AND ARTHUR L. BRUECKNER

Specific inclusion bodies present in the central nervous system of animals dying from rabies are called "Negri bodies" (Negri 1903–1907). These are most frequently demonstrated within, but occasionally outside, the nerve cell. A definite diagnosis of rabies is made upon demonstration of these bodies in stained smears. The inclusion are eosinophilic bodies, usually spherical, but of other shapes—varying from 1 to 30 microns in size. The inner structure shows eosinophilic granules which resemble virus-like particles seen in previous electron microscopic studies of Negri bodies (Reagan *et al.*, 1950–55). Demonstration of Negri bodies in the brain of an animal is diagnostic of the presence of rabies virus.

In the following experiment, the demonstration of Negri bodies was used as the basis for diagnosis of rabies in the animals, and neutralization tests were also used to identify the fixed strain and Flury strain as rabies virus. Electron microscopic studies of the rabies virus strain V308 (not the inclusion body) were reported in a previous paper (Reagan *et al.*, 1953), and the virus measured from 100 to 120 mu in diameter. The following experiment was undertaken to compare the difference between the Flury egg-adapted strain, a street-virus strain, and a fixed strain which had been carried in mice intracerebrally for hundreds of passages, the exact passage being unknown. The fixed strain showed an occasional Negri body upon mouse brain examination.

The rabies virus, strain R-174 Lederle, used in the present study was isolated in Swiss albino mice which had been inoculated with a brain suspension from a dog with rabies. This strain was obtained from Dr. H. R. Cox of the Lederle Laboratories Division, Pearl River, New York. This specimen had been proven

* From the Virus Laboratory, University of Maryland, College Park, Maryland. Received for publication January 18, 1955.

positive for rabies at the Lederle Laboratories. This specimen, received as a 20 per cent suspension, was reground in a mortar with alundum, and diluted to a 10 per cent suspension with physiological saline. It was then frozen in the —60° C. deep freeze storage vault until the initiation of the present study.

The rabies virus, strain C.V.S. sub-strain fixed virus, was obtained from Dr. Habel of the National Institutes of Health, Bethesda, Maryland. This is the standard test virus, original Pasteur strain. This specimen, upon arrival (10 per cent suspension) was also placed in the —60° C. deep freeze vault. Dr. Habel stated that this is the standard fixed virus strain used at the National Institutes of Health. (This fixed virus strain was also received from the Lederle Laboratories but was not used.) The Flury R-166-225th egg passage 40 per cent suspension was also received from Dr. Herald Cox of the Lederle Laboratories and was diluted to a 10 per cent suspension with physiological saline and used as is for this study.

### *Materials and Methods*

Twelve 4-week-old Swiss albino mice were inoculated intracerebrally with 0.03 cc. of a 20 per cent virus-bearing brain suspension of the first mouse passage street virus, strain Lederle R-174. Twelve 4-week-old Swiss albino mice were inoculated intracerebrally with the fixed virus likewise, and twelve 4-week-old Swiss albino mice were inoculated with 10 per cent normal brain suspension. Between five and eight days, Swiss mice from the street virus group showed symptoms of nervous system involvement. When symptoms appeared the mice were destroyed and the brain removed aseptically. Smear preparations from these animal brains were stained with Seller's stain (Seller, 1927), and upon examination under an optical light microscope, were found to contain numerous Negri bodies. Between five and nine days, mice from the fixed virus group showed similar symptoms and they were treated likewise; although, upon examination of these brains, only an occasional Negri body was seen. Mice from the control group appeared normal and they were also sacrificed after eight days. Upon examination of their brains no Negri bodies were seen. The pools of the infected mouse brains (street virus strain), of the infected mouse brains (fixed virus strain), of the normal mouse brains (injected intracerebrally with normal mouse brain eight days previously), of normal chick embryo suspension, of the Flury egg adapted embryo suspension were ground with alundum and diluted to 10 per cent suspension with physiological saline. The five suspensions were then subjected to centrifugation for five minutes in an angle centrifuge at 2,000 rpm. The supernatant fluids were removed from each of the five pools separately and filtered separately through type ST size L6 Seitz filters. The filtrates

were spun in a refrigerated Spinco ultracentrifuge at 44,770 rpm for two hours. The temperature of the refrigerated outer jacket remained constant at —10° C. during the centrifugation. The supernatants from the specimens were discarded and the sediment from each was resuspensed in 1.0 per cent physiological saline. Several drops of each of the five suspensions were placed on separate parlodion film supports which had been prepared seventy-two hours previously. After all excess fluid had been removed with a small capillary pipette, the films were dried and shadowed with chromium (Williams and Wyckoff, 1946) at arc tangent 2/10 and examined under the RCA electron microscope, Type E.M.U. The balance of the infected street virus material was injected intracerebrally in six Swiss mice and the balance of the infected fixed rabies virus suspension was done identically. The balance of the normal brain and chick embryo material was also injected intracerebrally into six Swiss mice each, and the balance of the Flury egg-adapted strain was injected into ten suckling Swiss albino mice.

## *Results*

Three of the six mice injected intracerebrally with the concentrated infected street virus material showed symptoms of furious rabies after a period of six days. The mice were destroyed and their brains contained numerous Negri bodies. Four of the six mice injected intracerebrally with the concentrated infected

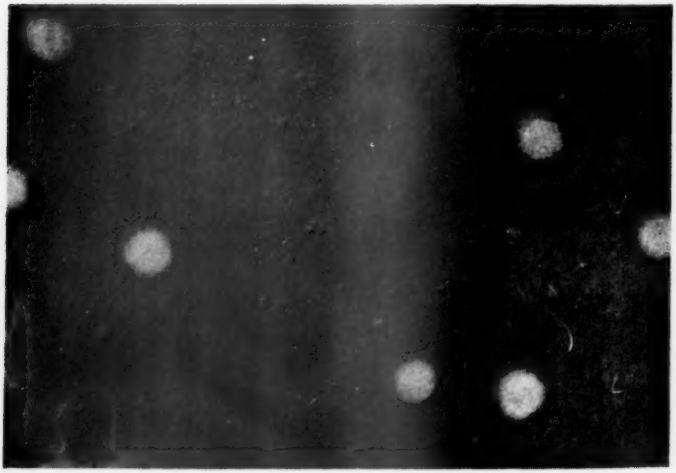

FIG. I. Electron Micrographs of Virus Particles from the Street Virus Rabies Strain (Lederle R-174) x 72,000 shadowed with chromium at tangent 2/10.

fixed virus material showed rabies symptoms after a period of eight days. (Probably the reason why not all mice showed symptoms from the three infected pools was because the virus of rabies is not readily filtrable through Seitz EK filter pads.) The mice were destroyed. The brains showed an occasional Negri body, and neutralization tests confirmed that the concentrate contained rabies virus. Five of the Flury-infected suckling mice showed rabies symptoms after ten days, but no Negri bodies were seen. The neutralization test in suckling mice confirmed the virus to be rabies. The Swiss mice injected with the concentrated suspension of normal mouse brain and normal chick embryo appeared normal and were discarded after a fourteen-day observation period. Upon electron microscopic examination of the concentrated suspension of the brain of the normal mice and normal chick embryo, no virus-like particles could be seen. Upon the examination of the concentrated suspension from the infected street virus mouse brains, virus particles were demonstrated as shown in figure I. These virus-like particles appear to be spherical with

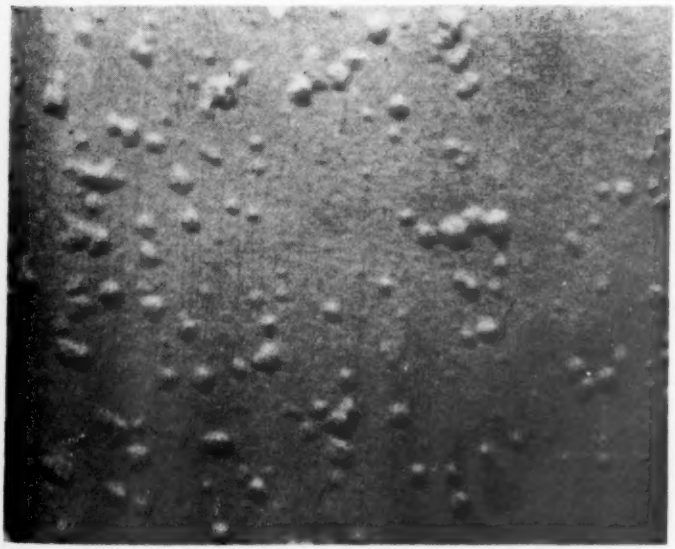

FIG. II. Electron Micrographs of Virus Particles from the Pasteur Fixed Virus Strain (Habel) x 55,000 shadowed with chromium at tangent 2/10.

irregular contour and with a diameter of 110 to 120 mu. A street virus strain described in a previous paper measured 100 to 120 mu (Reagan *et al.*, 1953) and upon examination of the concentrated suspension form the infected fixed virus mouse brains the virus also appears to be spherical with irregular contours and with a diameter of 80 mu as shown in figure II. The Flury strain as shown in figure III also appears to be spherical with irregular contours and with a virus diameter of 110 mu.

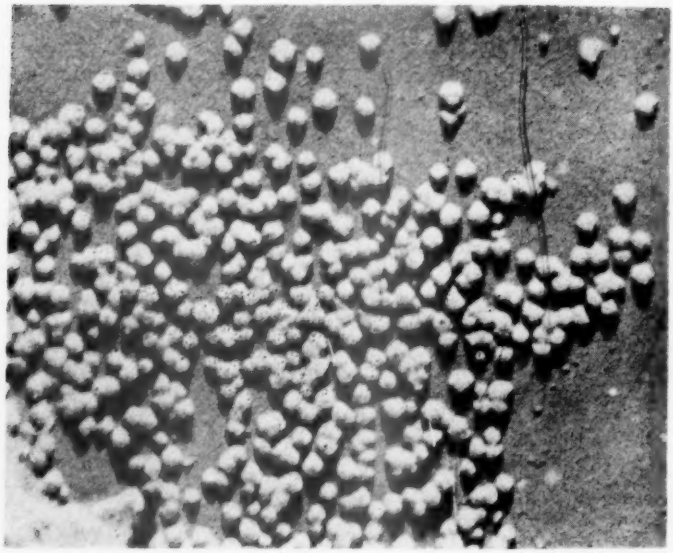

FIG. III. Electron Micrographs of Virus Particles from the Flury Egg-Adapted Strain (r-166 225th egg passage) x 40,000 shadowed with chromium at tangent 2/10.

*Summary.* Studies by the electron microscope of brains of mice infected with strain R-174 Lederle street rabies virus, showed the virus to vary from 110 to 120 mμ. In comparison, the study of brains of mice infected with the Pasteur N.I.H. Habel fixed-virus rabies strain measured 80 mμ in diameter, and the Flury egg adapted rabies strain measured 110 mμ in diameter. These bodies could not be demonstrated in concentrated normal mouse brain and normal chick embryo subjected to the same pro-

cedure of concentration and examination. The concentrated virus-bearing mouse street virus upon being injected into normal mice intracerebrally, produced rabies as did the fixed virus strain and the Flury strain which was injected into suckling mice intracerebrally. The concentrated material from normal mouse brains and normal chick embryo injected intracerebrally into Swiss mice produced no evidence of rabies. Neutralization tests with the Flury and fixed virus rabies strains confirmed the virus in the material examined to be rabies virus, while mouse brains infected with the street virus showed numerous Negri bodies and confirmed this strain to be rabies.

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# THE PANTOTHENIC ACID DEFICIENT RAT
## I. CHOLESTEROL BIOSYNTHESIS*

LELAND L. SMITH† and ROY B. MEFFERD, JR.

Current views on the biogenesis of the sterols from acetate implicate participation of acetyl coenzyme A (AcCoA) as an intermediate. A number of studies have dealt with sterol biosynthesis in organisms deficient in pantothenic acid (PA), and of necessity, in CoA, since cellular pantothenic acid is recognized as being bound in CoA. PA deficient yeast (low CoA levels) have impaired abilities to convert acetate to ergosterol (Klein and Lipmann, 1953a) which is reversible by enrichment with CoA (Hanahan and al-Wakil, 1952). Liver slices from PA deficient rats were low in CoA and also incorporated less acetate into cholesterol (Klein and Lipmann, 1953b). Generally lower concentrations of cholesterol are found in the liver (Klein and Lipmann, 1953b; Guehring *et al.*, 1952) and in the adrenals (Winters *et al.*, 1952; Dumm *et al.*, 1953; Perry *et al.*, 1953; Boyd, 1953) of P A deficient rats.

With respect to the rate of incorporation of labeled acetate into cholesterol and fatty acids, maximal incorporation of radioactivity in the intact animal following a single intraperitoneal (*i.p.*) injection of acetate-1-$C^{14}$ occurs very rapidly (Van Bruggen *et al.*, 1953; Ruyssen *et al.*, 1951; Hevesy *et al.*, 1951, 1952). The presence of radioactive impurities in the digitonin-precipitable fraction of the intact rat shortly after acetate injection and their gradual disappearance over a period of several hours has also been reported (Schwenk and Werthessen, 1953). This report covers the results of an investigation of sterol biosynthesis in the intact PA deficient rats.

### *Experimental*

*Care of Animals.* Young male and female Sherman-derived strain rats, averaging 118 g. in weight, were segregated into

* From Southwest Foundation for Research and Education and Trinity University, San Antonio, Texas. Supported in part by funds from the San Antonio Junior League and from National Muscular Dystrophy Research Foundation, Inc.

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three groups. Each group was maintained on a synthetic diet deficient in pantothenic acid[1] and kept in false-bottom cages. Two of the groups received 200 μg. of calcium pantothenate in 1 ml. of 0.85 per cent saline, *i.p.* every 48 hours.

The third group (the PA deficient animals) consumed an average of 12 g./day of the diet over a five-week period. Twenty-four hours prior to the tracer experiments the deficient animals were randomly segregated into two equal groups. Both of these received an *i.p.* injection of 1 ml. of 0.85 per cent saline with 200 μg. of calcium pantothenate being added in the second group. The latter is referred to as the "reversed" group.

Of the two groups receiving PA one was fed *ad libitum*, while the other was pair-fed with the deficient animals, *i.e.*, those receiving no pantothenate injections. All animals were weighed weekly.

After five weeks on the deficient diet classic signs of PA deficiency in the deficient group began to appear, first a slowing in weight gain, then the signs of alopecia, and of porphyrin-caked whiskers and nose. Severe deficiency symptoms were not manifest at this time. In an earlier experiment where severe symptoms were allowed to develop, highly erratic results were obtained. In such a situation complex multiple deficiencies are actually present, further complicated by inanition. All animals in the two control groups were healthy in appearance and were gaining weight steadily, although not as rapidly as equivalent animals did on Purina Lab. Chow.

*Tracer Experiment.* Animals from each group were withdrawn randomly, segregated, and weighed. Sodium acetate-1-$C^{14}$ (160 μc./mg.)[2] was injected *i.p.* (1 μc./g.; 6.25 μg./g.), and the animals were sacrificed at intervals thereafter by a sharp blow on the head. Immediately after sacrifice equal weights of potassium hydroxide pellets and of water were added to the carcass, and the mixture warmed on a steam bath overnight. The cooled hydrolysate was diluted with a half volume of water and ex-

[1] Obtained from Nutritional Biochemicals Corporation, Cleveland, Ohio. The diet consisted of vitamin test casein, 18 per cent; sucrose, 68 per cent; vegetable oil, 10 per cent; USP salt mixture No. 2, 4 per cent. In addition the diet was fortified with: (g./100 lbs. diet) vitamin A concentrate, 4.5; vitamin D concentrate, 3.0; α-tocopherol, 10.25; inositol, 13.7; choline chloride, 273.0; menadione, 0.2; niacin, 27.25; *p*-aminobenzoic acid, 1.0; riboflavin, 1.0; pyridoxine HCl, 1.0; thiamine HCl, 1.0; biotin, 0.03 mg./100 lbs.; folic acid, 0.01 mg./100 lbs.

[2] Prepared from barium carbonate-$C^{14}$.

tracted exhaustively with pentane, and the washed extracts were evaporated under nitrogen. The digitonin-precipitable fraction was isolated by the standard methods (Schwenk *et al.*, 1952) and regenerated with pyridine-ether. The sterols were purified via the dibromide, and the pure cholesterol was recrystallized from methyl alcohol. All techniques were highly standardized so as to yield as nearly comparable results as possible.

To test the rapidity with which an animal might be injected, sacrificed, and hydrolyzed, an animal from each group was injected with acetate, immediately killed, and processed as usual. The total time elapsing from injection to mixing of water and alkali with the carcass was about one minute. Virtually complete disintegration of the carcass occurred within five minutes.

Two non-deficient female rats from the *ad libitum*-fed control group were withdrawn separately and injected with 200 μg. of calcium pantothenate in saline 30 minutes prior to injection with acetate. Thirty minutes after the injection of acetate they were sacrificed and processed.

*Radioactivity Analyses.* Cholesterol from each animal was plated onto disks as previously described (Smith and Werthessen, 1953). Weights of the samples varied from 1.8 to 2.2 mg. Samples were counted with a 2 mg./sq. cm. geiger tube and a Tracerlab 100 scaler. Sufficient counts were totaled to assure a statistical error of less than 5 per cent. All analyses were run in duplicate, and the specific activities, corrected for self-absorption, were averaged.

## *Results*

The data presented in table 1 summarize the results obtained in the experiments conducted with PA deficient male and female rats. The average weight variations of the animals over the five-week period while on the diet are recorded in figure I. These trends are characteristic of PA deficiency (Klein and Lipmann, 1953b) and are presented to establish the likelihood of an actual PA deficiency in the test animals.

The relationship between total sterol content (digitonin precipitated material) and body weight is of interest. The data plotted in figure II illustrates the trend involved: light animals have high sterol content while heavier animals tend to have

lower and less variable sterol content. Other investigations have indicated lower sterol concentrations in liver and adrenals of PA deficient rats.

These results clearly indicate that the opposite situation is the case in the intact animal. These data may be explained in part in that it may be more difficult to extract the sterols from the

TABLE 1

*Cholesterol Biosynthesis in Pantothenic Acid Deficient Rats*

| Time After C-14 Injection, Minutes | Sex | Final Weight, g. | Calc. Weight Sterols, mg. | Sterols in Rat Per cent | Specific Activity, Counts/min./mg. Purified via: Digitonin | Digitonin, Dibromide | RIC |
|---|---|---|---|---|---|---|---|
| *Control Animals, Fed ad libitum:* | | | | | | | |
| 0 | M | 298 | 147 | 0.049 | 0 | 0 | 0 |
| 15 | M | 248 | 101 | 0.040 | 107 | 35 | 0.13 |
| 15 | F | 190 | 128 | 0.067 | 195 | 73 | 0.46 |
| 30 | M | 244 | 144 | 0.059 | 310 | 174 | 0.96 |
| 30 | M | 242 | 211 | 0.087 | 375 | 127 | 1.03 |
| 60 | F | 228 | 93 | 0.041 | 444 | 277 | 1.06 |
| 90 | M | 233 | 150 | 0.064 | 360 | 223 | 1.34 |
| 120 | M | 238 | 212 | 0.089 | 415 | 269 | 2.24 |
| *Control Animals, Restricted Feeding:* | | | | | | | |
| 0 | M | 222 | 26 | 0.012 | 0 | 0 | 0 |
| 15 | M | 260 | 200 | 0.077 | 63 | 31 | 0.22 |
| 15 | F | 196 | 70 | 0.036 | 150 | 34 | 0.11 |
| 30 | M | 235 | 234 | 0.099 | 165 | 53 | 0.49 |
| 30 | F | 178 | 126 | 0.071 | 284 | 148 | 0.98 |
| 60 | M | 270 | 193 | 0.071 | 282 | 92 | 0.61 |
| *Deficient Animals:* | | | | | | | |
| 0 | M | 199 | 175 | 0.088 | 0 | 0 | 0 |
| 15 | M | 120 | 236 | 0.197 | 206 | 123 | 2.27 |
| 15 | F | 107 | 189 | 0.176 | 216 | 105 | 1.69 |
| 30 | M | 136 | 176 | 0.129 | 232 | 112 | 1.32 |
| 30 | F | 99 | 265 | 0.268 | 217 | 127 | 3.15 |
| 60 | M | 163 | 279 | 0.171 | 182 | 83 | 1.37 |
| 60 | F | 116 | 276 | 0.238 | 300 | 227 | 4.89 |
| 90 | M | 164 | 225 | 0.137 | 305 | 120 | 1.58 |
| 90 | F | 116 | 219 | 0.189 | 155 | 101 | 1.72 |
| 120 | M | 176 | 205 | 0.116 | 232 | 162 | 1.73 |
| *Deficiency-Reversed Animals:* | | | | | | | |
| 0 | M | 159 | 193 | 0.121 | 0 | 0 | 0 |
| 15 | M | 156 | 221 | 0.142 | 202 | 63 | 0.83 |
| 15 | M | 179 | 59 | 0.033 | 232 | 79 | 0.24 |
| 30 | M | 189 | 149 | 0.079 | 287 | 198 | 1.46 |
| 30 | M | 170 | 234 | 0.138 | 410 | 207 | 2.66 |
| 60 | M | 142 | 228 | 0.161 | 425 | 170 | 2.55 |
| 60 | M | 177 | 131 | 0.074 | 315 | 262 | 1.81 |
| 90 | M | 164 | 110 | 0.067 | 476 | 345 | 2.17 |
| 90 | M | 172 | 228 | 0.133 | 319 | 236 | 2.92 |
| 120 | M | 174 | 289 | 0.166 | 444 | 215 | 3.34 |

M denotes male.
F denotes female.

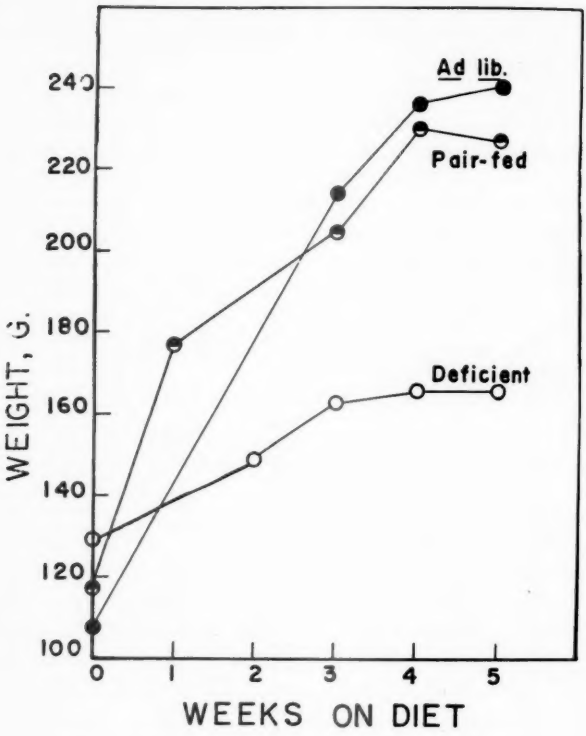


FIG. I. Average weight gain of deficient, pair-fed, and *ad libitum*-fed rats.

larger animals, but we feel this is not the major consideration. The differences between the average sterol content of the deficient and of the control groups are statistically significant ($p<0.01$, Student's "t" test), while that of the two controls are not. The individual variation is markedly increased by either a caloric restriction or a PA deficiency. These were not highly inbred rats, and there is, therefore, considerable variation between individuals with respect to any chosen criterion. We feel that most of the variability is explainable in genetic terms, and that the chemical errors contribute only a fraction of the total variation. As metabolism is increasingly disrupted dietetically (re-

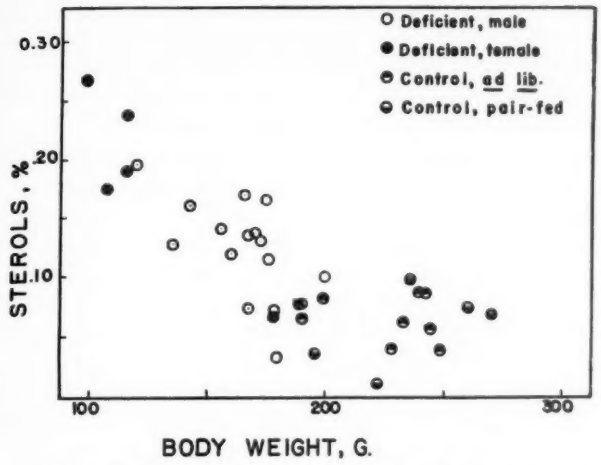


FIG. II. Variation in sterol (digitonin precipitated) content of PA deficient and non-deficient rats in relation to total body weight.

stricted diet, or PA deficiency) an increasing variability was noted. Since animals fed *ad libitum* on the synthetic diet gained less than animals on Purina Lab. chow, it is reasonable to believe that variability would probably have been lower in the latter. Biochemical individuality of PA deficient rats will be reported in a later communication (Mefferd, Smith, Martens, and Wilson, in preparation).

No pronounced difference in isotope incorporation behavior was noted in the two sexes. The highest sterol content is associated with females only in that they were lighter animals. Though the animals were treated individually and sex is noted, average values were used in the several conclusions drawn from the data.

*Discussion.* The results obtained on the radioactivity analyses of the digitonin-precipitable material (sterol fraction) and of the cholesterol therefrom (purified via the dibromide to radiochemical purity) indicate that the incorporation of acetate-1-$C^{14}$ into these fractions is definitely altered in the PA deficient animals. Lata and Anderson (1954) recently reported similar observations for liver slices of PA-deficient rats.

There are several criteria to be considered in these experiments. These points are: time course of specific activity, time course of loss in specific activity on purification from the digitonin precipitated sterols to cholesterol purified via the dibromide, and time course of relative isotope concentration.

From table 1 considerations of the specific activities of the impure and pure cholesterol fractions indicate rapid attainment of maximum specific activities, followed by little change over the period studied. Although the level of maximum specific activity for the deficient group is considerably less than that for the *ad libitum*-fed and reversed groups, a distinct decrease in the rate of incorporation is reached by 15 minutes after acetate administration, while the other groups exhibit such changes after 30 minutes. This rapid attainment of maxima or plateaus in specific activities of cholesterol isolated from the intact rat and mouse has been previously reported (Van Bruggen *et al.*, 1953).

The specific activity of the impure cholesterol fraction is invariably greater than that of the pure cholesterol. This loss in count has been suggested as being caused by removal of highly radioactive precursors from the cholesterol purified via the dibromide (Schwenk and Werthessen, 1952, 1953). The exact nature of these radioactive contaminants, the higher counting companions (HCC) of Schwenk and co-workers, is obscure.

An initial loss of 65 per cent in the control and reversed groups, followed by a decrease to 40 per cent loss in count is consistent with the interpretation that certain portions of the radioactive contaminants are precursors of cholesterol. (Other interpretations are possible.) The absence of a high level per cent loss in PA deficient group might then be indicative of the absence of certain radioactive precursors. The constant loss in the pair-fed control group at 65 per cent levels over the first 60 minutes is then possibly indicative of slower conversion into cholesterol of the precursors and may represent an accumulation of radioactive precursors. Figure III presents data illustrating these trends.

Six hours following the acetate injection the specific activity of the cholesterol isolated via digitonin alone and of cholesterol further purified via the dibromide was essentially the same (134 and 137 c./min./mg. for PA deficient rats, 198 and 172 c./ min./

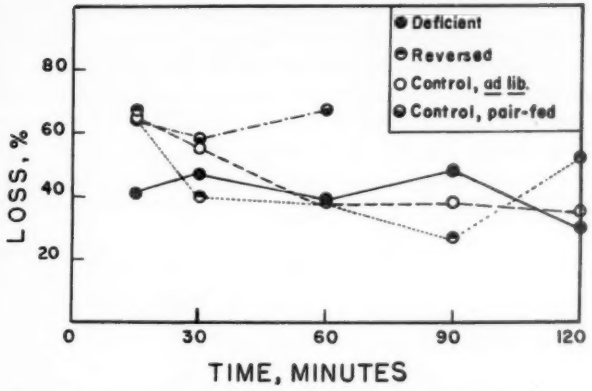


FIG. III. The per cent loss in specific activity incurred by purifying the digitonin precitable sterols via the dibromide.

mg. for controls, 204 and 165 c./min./mg. for reversed deficiency, respectively).

Consideration of the relative isotope concentration,[3] defined as:

$$\text{RIC} = \frac{(\text{counts/min./mg.}) \times (\text{mg. cholesterol}) \times 10^{-5}}{(\text{mc. acetate injected})}$$

permits more impressive conclusions in regard to the effect of PA deficiency on sterol biogenesis. The RIC takes into account the cholesterol masses in the different groups of rats, this being important in view of the cholesterol content-weight relationship disclosed earlier. The RIC may be considered as the isotope incorporation corrected for the quantity of sterol present rather than corrected on a total weight basis. The RIC is essentially a relative yield or conversion of acetate to cholesterol.

Consideration of RIC data indicates the same initial rapid rises followed by a slower rise in the case of the *ad lib.*-fed controls and reversed animals, but with a maximum of 60 minutes for the PA deficient animals. The ratios of the RIC of the experi-

[3] Our laboratory is not equipped to determine "radioactive yield," and we prefer to use the term "relative isotope concentration" rather than the "efficiency factor" (EF) used by some workers so as to avoid the implication of more knowledge about the data than is actually available. The RIC is $10^{-1}$ of the EF, as defined by Schwenk and Werthessen.

mental groups to those of the *al lib.*-fed controls, presented in figure IV bring the data into their final form.

Given the average maximum specific activity and the average percentage sterol the RIC ratios of the several groups may be

TABLE 2

*Comparison of Relative Isotope Concentration, Predicted and Observed*

| Group | (A) Average Specific Activity at Max., c/min./mg. | (B) Average Sterol Content, Per cent | A X B* | Average RIC Ratio, Predicted | Average RIC Ratio, Observed |
|---|---|---|---|---|---|
| Deficient | 132 | 0.173 | 22.8 | 1.6 | 6.5 |
| Reversed | 231 | 0.111 | 25.6 | 1.8 | 1.9 |
| Control, pair-fed | 96 | 0.061 | 5.8 | 0.4 | 0.6 |
| Control, *ad lib.*-fed | 230 | 0.062 | 14.4 | 1.0 | 1.0 |

* The product A X B, is proportional to the RIC defined in the text. The ratio of the product for the experimental groups to that of the *ad lib.*-fed controls gives the predicted RIC ratios.

predicted as in table 2. The average maximum specific activity is taken from the period of maximum constant specific activity (15 to 120 minutes for the PA deficient groups, and 30 to 120 minutes for the other groups). The average sterol content is taken as the average of all values determined for each group. The product of these two values is proportional to the RIC value, since the percentage of sterol involves a term composed of the weight of sterol per unit weight of rat. The latter was for the administration of acetate-1-$C^{14}$ in each case. The ratio of this specific activity-per cent sterol product between the experimental groups and the *ad lib.*-fed control group is, therefore, the predicted RIC ratio for rats of similar weights to the average weights of the animals of a particular experimental group over the period of relatively constant maximum specific activity of the sterol fraction.

The predicted RIC ratios of table 2 should agree with those determined experimentally. This is the case in the reversed group and the pair-fed control group. The fourfold increase over the predicted value for the deficient group at 15 minutes followed by a decrease to values lower than predicted implies deep-seated alterations in acetate metabolism in PA deficient rats.

It would appear that PA deficient rats utilize acetate for cholesterol much more rapidly than do non-deficient animals imme-

diately following an intraperitoneal injection of tracer amounts of acetate-1-$C^{14}$. This rapid utilization decreased within 90 minutes to much lower levels. This observation strengthens the suggestion that acetate formation as well as CoA function in PA deficient rats is abnormal (Riggs and Hegsted, 1948). These authors demonstrated that the impaired acetylation of *p*-aminobenzoic acid in PA deficient rats was improved by feeding acetate.

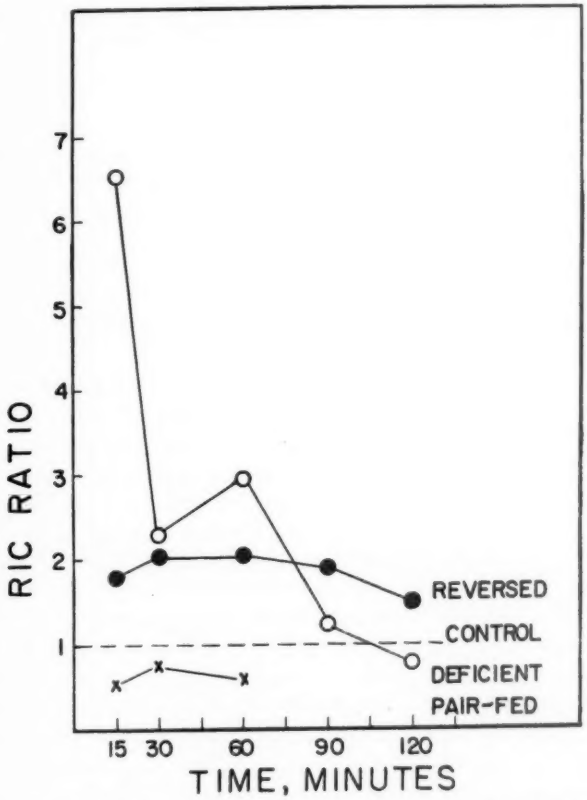


FIG. IV. Variation in RIC of the PA deficient animals, pair-fed controls, and reversed animals relative to the RIC of the *ad libitum*-fed controls.

Rats deficient in PA and therefore in CoA function may have unsatisfied demands for acetate which cannot be met due to an impaired ability to initiate the Krebs cycle. Injection of even tracer amounts of acetate-1-$C^{14}$ may present a temporary excess or "prime" to the metabolically disturbed system and possibly acetate-depleted organism, which then responds by rapid metabolic activity involving both energy and synthetic mechanisms. Thus the specific activity of the cholesterol fraction rises to a maximum within 15 minutes and there are smaller losses in specific activity (HCC) in the purification processes, possibly due to increased utilization of precursors for cholesterol biosynthesis. As the temporary excess of acetate or "prime" is depleted by the increased metabolic activity itself, the rate of incorporation of acetate into cholesterol would then decrease to levels inordinately lower than predicted, in the presence of the highly competitive energy-yielding systems. It is reasonable to believe that the various apoenzymes requiring CoA may have different degrees of avidity for the coenzyme. If this be true, then various enzyme systems would exhibit different states of impaired function during the development of the PA deficiency, and it would be expected that utilization of the available acetate would be the subject of altered competitive patterns.

The enzymes clinging tenaciously to their CoA would be in a better competitive position than those which suffer in the competition for available CoA. The ramification of a PA deficiency may well extend much further than mere competition for coenzyme and substrate. It is probable that all enzymes are basically adaptive in nature, *i.e.*, their formation is stimulated by the presence of the specific substrate, and that each enzyme-synthesizing mechanism has a definite potentiality in this respect. An over-all deficiency of a substrate required by several systems, ***e.g.***, CoA, would then be expected to result in a differential synthesis of the various apoenzymes requiring CoA. A near-constitutive enzyme would be less affected by a deficiency than would be a highly adaptive one, and any given degree of deficiency would result in altered proportions of the various CoA enzymes. Although much information must be gained before these considerations may be adequately evaluated (*e.g.*, avidities, activities, and actual concentrations of the various CoA-enzymes present in

various degrees of PA deficiency) it is now clear that a PA deficiency results in altered acetate metabolism, and this to widely varying degrees for various systems (Smith and Mefferd, 1955).

Sterol degradative processes must also be impaired in the PA deficient intact rat, as evidenced by the high percentages of sterol present in these animals. High cholesterol levels in plasma have been noted in PA deficient rats (Perry *et al.*, 1953) although lower levels prevail in the adrenals and liver of such animals.

Chronic caloric restriction such as is present in the pair-fed group, results in a 40 per cent decrease in acetate utilization for cholesterol synthesis. These findings agree with similar results obtained from fasted rats. After 120 hours of fasting rats which had lost 24 per cent of their body weight and 75 per cent of their fatty acids maintained their cholesterol masses, although the amounts of isotope incorporated from an *i.p.* injection of acetate-1-$C^{14}$ were reduced (Van Bruggen *et al.*, 1952). Other workers found that expired $CO_2$ in such animals contained reduced amounts of isotope incorporated following acetate injection (Coniglio *et al.*, 1952). Studies with liver slice incubations indicate the same decreased capacity to incorporate acetate into cholesterol and fatty acids in fasted rats (Lyon *et al.*, 1952; Tomkins *et al.*, 1952).

The reversed deficient group and the *ad lib.*-fed control group present identical behavior as regards the several criteria imposed. It would appear that the injection of 200 μg. of calcium pantothenate 24 hours prior to acetate injection actually did reverse the PA deficiency as regards cholesterol synthesis. PA deficiencies have been ameliorated in ducks and rats by prior injection of calcium pantothenate, and impaired pyruvate utilization and acetylation of sulfanilamide and *p*-aminobenzoic acid were restored to control levels in the animals (Riggs and Hegsted, 1948; Olson and Kaplan, 1948; Shils *et al.*, 1951). The physical appearance of our reversed animals did not improve over the 24-hour period, and the high per cent of sterols also was unaltered.

The effects of an intraperitoneal injection of 200 μg. of calcium pantothenate 30 minutes prior to acetate injection was investigated in non-deficient females. In table 3 is presented information on these experiments. A slightly decreased incorpora-

TABLE 3

*Effect of Injection of Calcium Pantothenate on Sterol Biosyntheses**

| Animal Weight, g. | Calc. Weight Sterols, mg. | Sterols Per cent | Specific Activity, counts/min./mg. Purified via Digitonin | Dibromide | RIC |
|---|---|---|---|---|---|
| 190 | 149.0 | 0.078 | 158 | 80 | 0.58 |
| 198 | 167.0 | 0.084 | 140 | 81 | 0.64 |

* Both animals, females, were injected intraperitoneally with 200 μg. of calcium pantothenate in 1 ml. of 0.85 per cent saline 30 minutes prior to injection of acetate-1-$C^{14}$. Following acetate injection 30 minutes was allowed to elapse before sacrifice.

tion of acetate carbon into cholesterol prevailed. Migicowsky and Greenberg (1954) found that CoA inhibited the conversion of acetate to cholesterol in certain rat liver homogenate experiments.

*Summary.* Following a single intraperitoneal injection of sodium acetate-1-$C^{14}$ a maximum in isotope incorporation into cholesterol occurs within 15 minutes in the PA deficient rat, and at 30 minutes in the non-deficient rat. The specific activities show little change over two hours once having reached their maxima. The PA deficient animal has four times the relative isotope content of non-deficient controls 15 minutes following acetate injection; however, this increased RIC decreases to low levels after 90 minutes.

PA deficient rats injected with calcium pantothenate 24 hours prior to acetate injection have identical patterns of isotope incorporation into cholesterol with non-deficient controls. Injection of calcium pantothenate 30 minutes prior to acetate injection results in a slight lowering of isotope incorporation into cholesterol.

PA deficient rats contain a significantly higher per cent of total body sterols than do non-deficient rats.

Rats pair-fed with PA deficient animals had about 60 per cent the capacity of acetate conversion to cholesterol as did rats fed *ad libitum*.

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# CALLING ATTENTION TO:

## Items of Possible Interest to Our Friends

### March 1955

General: D Bleitz does superb job of color photography of birds *(Arizona Highways*, Mch/55) . . . .WEL Clark gives *The Fossil Evidence for Human Evolution* (Univ Chicago Press, 55, 196pp, $6) . . . .NB: Justice WO Douglas, *Almanac of Liberty* is OK (Garden City, 55, 402pp, $5) . . . .HM Fox analyses the colours of animals *(Endeavor* 14/40/55) . . . . PC Glick analyses the life cycle of the family *(Marriage & Family Living* 17/3/55) . . . . P Greenacre surveys pathography of Jonathon Swift (1667–1745) (Psychoanal Quart 24/20/55) . . . CC Li considers *Population Genetics* (Univ Chicago Press, 55, 440pp, $15) . . . .G Liljestrand & Y Zotterman describe water taste in mammals *(Acta Physiol Scand* 32/291/54) . . . . F Mainx reviews the *Foundations of Biology* (Univ Chicago Press, 55, 104pp, $2) . . . . ED Merrill gives delightful account of botany of Cook's voyages (1768–1780) & unexpected significance in anthropology, biogeography, & history (*Chronica Bot* 14/164–384/54). . . . O Riddle reveals *The Unleashing of Evolutionary Thought* (Vantage Press, NY, 54, 414pp, $4.5) . . . . WE Ritter considers *Charles Darwin & The Golden Rule* (Science Service, Washington, 54, 400pp, $5) . . . .C Singer tells about Robert Hooke (1653–1703), first English microscopist *(Endeavor* 14/12/55) . . . . NB: Symposium on science as social & historical phenomenon by H Guerlac, EG Boring, A Koyre, RS Cohen *(Sci Month* 80/93–116/55) . . . . HW Smith edits important conference on law & medicine *(J Publ Law* 3/288–736/55) . . . . EN Willmer discusses the 12 white colors *(Endeavor* 14/19/55) . . . .

Socio-psychiatry: BK Anand & Co explore hypothalamic influence on food intake & on pituitary-adrenal cortex activity *(J Physiol*, 127/143, 153/55) . . . .HW Brosin analyses discovery & experiment in psychiatry (*Amer J Psychi* 111/561/55) . . . .EC Dax discusses value of social activities in mental hospital treatment (*Med J Austral* 1/25/Jan 8/55). . . . HD Fabing describes anti-psychotic action of gamma piperidyl-dibenz-hydrol (*Science* 121/208/Feb 11/55). . . . D Goldstine edits *Expanding Horizons in Medical Social Work* (Univ Chicago Press, 55, 288pp, $5). . . . LB Hill considers *Psychoterapeutic Intervention in Schizophrenia* (Univ Chicago Press, 55, 236pp, $5). . . . HB Peck, MR Harrower & Co report mental health services in a Children's Court (*Am J. Orthopsychi* 25/1/55). . . . WR Perl indicates psychodiagnostic value of handwriting analysis (*Am J Psychi* 111/595/55). . . . JF Tuthill well analyses impotence (*Lancet* 1/124/Jan 15/55). . . .

Clinical: HL Alexander notes *Reactions with Drug Therapy* (Saunders, Phila, 55, 301pp, $7.5). . . . JA Arona Jr & A Blain find rapid thawing increases injury in frost-bite (*Surg* 37/175/55) . . . . LA Calkins describes *Normal Labor* (CC Thomas, Springfield, Ill, 55, 128pp, $2.5). . . . MAC Dowling reviews insect repellants & miticides (*J Roy Army Med Corps*

101/1/55). . . . JS Fish considers *Hemorrhage of Late Pregnancy* (CC Thomas, Springfield, Ill, 55, 180pp, $4). . . . AW Hobby surveys pathology & management of cough (*Am J Surg* 89/285/55). . . . WSS Landell discusses water & salt intake in workers in hot humid climates (*J Physiol* 127/11/55). . . . PM McAllen shows potassium deficiency causes myocardial fibrosis (*Brit Heart J* 17/5/55). . . . B Russel & NA Thorne review skin reactions from adhesive plasters (*Lancet* 1/67/Jan 8/55). . . . M Santer & OC Durham survey *Regional Allergy of the United States, Canada, Mexico & Cuba* (CC Thomas, Springfield, Ill, 55, 393pp, $7). . . . MS Segal & E Attinger note advances in inhalation therapy for chest diseases (*Am J Surg* 89/387/55).

PRECLINICAL: Sir MacFarlane Burnet discusses riddle of influenza virus (*Endeavor* 14/5/55). . . . EA Carr surveys drug allergy (*Pharmacol. Rev.*, 6/365/54). . . . JD Davidson & BB Freeman find purine antimetabolites depress nucleic acid synthesis in tumors (*Cancer Res* 15/31/55). . . . Our GA Emerson & JD Archer note medicolegal aspects of pharmacology (*J Publ Law* 3/393/54). . . . V Erspamer studies fate of indole alkylamines in organisms (*J Physiol* 127/118/55). . . . WB Healy & Co show similarity in iron deficiency & cobalt toxicity effects on enzyme systems (*Arch Biochem Biophys* 54/206/55). . . . CDL reviews drug allergies (*Postgrad Med* 17/132/55). . . . SL Palay & GE Palade describe fine structure of neurons (*J Biophys Biochem Cytol* 1/69/55). . . . A Takka studies sulfhydryl groups in crystalline lens in relation to cateractogenesis (*Acta Path Microbiol Scand* 36/21/55). . . . WN Valentine & Co. indicate basophils are chief histamine carriers in blood (*Blood* 10/154/55).

APRIL 1955

CLINICAL: HR Bierman enjoys oft-found but ill-defined Yoo-Yoo's Disease (*New Eng J Med* 252/274/Feb 17/55). . . . LJ Cole & Co study radiation protection factor in spleen (*Radiol* 64/201/55). . . . HW Florey reviews relation of lipids to atherosclerosis (*Med J Austral* 1/89/Jan 22/55). . . . JD Hardy describes *Fluid Therapy* (Lea & Febiger, Phila, 55, 255pp, $5.5). . . . SH Macht & PS Lawrence survey congenital defects from X-rays (*Am J Roentgen* 73/442/55). . . . P Malis & HL Schmiederer find amono-acid-electrolyte complex ("Zentramin") increases capillary resistance & limits damage from dicoumerol (*Arzneimittel Forsch* 5/30/55). . . . PJ Moloney & M Coval note antigenicity of insulin with diabetes induced by specific antibodies (*Biochem J* 59/179/55). . . . FJ Nattrass differentiates polymyositis (improved by ACTH) from muscular dystrophy (*Brain* 77/549/55). . . . JF Wade & JF Burnum fonfirm SP Beesman & Co (*Science* 117/659/53) on value of disodium calcium ethylenediamine tetra-acetate (versinate) as chelating agent in treating acute & chronic lead poisoning (*Ann Int Med* 42/25/55). . . . PD White gives *Clues in the Diagnosis & Treatment of Heart Disease* (CC Thomas, Springfield, Ill, 55, 186pp, $4.5). . . . World Health Organization issues helpful symposium on *Poliomyelitis* (WHO, Geneva, 55, 408pp). . . . R Zeledon describes trypanosomiasis rangeli (*Rev Biol Trop* 2/231/54).

GENERAL: E Becker edits *Financing Hospital Care in US, Vol 2: Prepay-*

*ment & Community* (McGraw-Hill, NY, 55, 356pp, $5). . . . MM Belli analyses *Modern Trials* (Bobbs-Merrill, Indianapolis, 55, 3 vols, $50). . . . WR Bett edits *History & Conquest of Common Diseases*, Univ Oklahoma Press, Norman, 55, 334pp, $4). . . . JT Bormer offers pattern for *Cells and Societies* (Princeton Univ Press, 55, 230pp, $4.5). . . . WR Brode well reviews color & chemical constitution (*Am Sci* 43/259/55). . . . WG Hogt & WB Langbein describe US *Floods* (Princeton Univ Press, 55, 352pp, $7.5). . . . WR Horsfall surveys *Mosquitoes: Their Bionomics & Relation to Disease* (Ronald, NY, 55, 723pp, $16). . . . WM Krogman considers Sherlock Holmes an anthropologist (*Sci Month* 80/155/55). . . . M Moore is stimulating in *Verse-Diary of a Psychiatrist* (*Contemporary Poetry*, Baltimore 10, 55, 39pp, $3). . . . E Teller tells many people helped develop nuclear energy (*Science* 121/267/Feb 25/55). . . . CE Whitmore discusses language of science (*Sci Month* 80/185/55). . . . CW Shilling describes *The Human Machine* (US Naval Academy, Annapolis, Md, 55, 292pp, $5).

PRECLINICAL: H Banner & A Muralt find succinodinitril stimulates nucleoprotein metabolism in cell nucleus & aids nerve cell regeneration (*Arch ges Physiol* 260/216/55). . . . EG Boring reviews nature & history of control in experimental studies (*Am J Psychol* 67/573/55). . . . A Cerletti & A Fauchamps discuss *L'Hibernation Artificielle* (Masson, Paris, 54) by H Labout (*Schw Med Wchnschr* 84/141/Feb 12/55). . . .LA Crandall Jr edits Symposium on *Sedative &Hypnotic Drugs* (Wms & Wilkins, Balt, 54, 111pp, $3.5). . . . JH Gaddum & Co study indole antagonists to hydroxy tryptamine (*Quart J Exp Physiol* 40/49/55). . . . FW Gairus describes sensory nerve endings of human palate (*Ibid* p 40). . . . A Goth & Co note role of amino acids in hormone secretion (*Experientia* 11/27/Jan 15/55). . . . JD Hopke reviews neuromuscular blocking agents (*J Anesth* 16/91/55). . . . RA Huggins & JH Moyer survey action of N-allyl normorphine (*Ibid* p 82). . . . J Kalin reviews phyletic significance of higher primates (*Experient* 11/1/Jan 15/55). . . . LC Katz edits symposium on cardiac regulation (*Physiol Rev* 35/91–168/55). . . . K Kuriaki & T Uchida suggest antihistaminics inhibit amine oxidase thus sensitizing peripheral organs to epinephrine & sympathetic stimulation (*J Pharmacol* 113/228/55). . . . HJ Kuschke & J Frantz note hyperglycemic action of reserpin (*Arch Exp Path Pharmakol* 224/269/55). . . . AS Marrazzi & ER Hart note relation of hallucinogens to adrenergic compounds (*Science* 121/365/Mch 11/55). . . . FC McLean & MR Urist consider *Bone: An Introduction to Study of Skeletal Tissue* (Univ Chicago Press, 55, 196pp, $6). . . . R Meier & Co note cell metabolism action of cytotoxic agents (*Arch Exp Path Pharmakol* 224/206/55). . . . RC Mellors writes *Analytic Cytology: Newer Methods for Studying Cellular Form & Function* (McGraw-Hill, NY, 55, 9 sections, $15). . . . LF Nims edits Symposium on Ironizing Radiation & the Cell (*Ann NY Acad Sci* 59/467–664/55). . . . RJ Ralls & BW Halstead discuss moray eel poison (*Am J Trop Med Hyg* 4/136/55). . . . E Racker surveys action of pyridine nucleotid-linked anzymes (*Physiol Rev* 35/1/55). . . . BO Rogers edits symposium on immunology & tissue homotransplantation (*Ann NY Acad Sci* 59/277–466/55). . . . R Tupper &

Co find zinc increase in tumors (*Biochem J* 59/264/55). . . . **GB West** reviews adrenalin & noradrenalin (*J Pharm Pharmacol* 7/81/55). . . . **CP Whittingham** discusses energy transformation in; photosynthesis (*Biol Rev* 30/40/55).